Antiviral strategies for hepatitis E virus

*Yannick Debing, Johan Neyts**

Rega Institute for Medical Research, Department of Microbiology and Immunology, Minderbroedersstraat 10, 3000 Leuven, Belgium

* Corresponding author:

Johan Neyts

Rega Institute for Medical Research

Department of Microbiology and Immunology

Minderbroedersstraat 10

3000 Leuven

Belgium

Tel. 32-16-33.73.41

Fax. 32-16-33.73.40

Email: johan.neyts@rega.kuleuven.be

ABSTRACT

The hepatitis E virus is a common cause of acute hepatitis. Contrary to hepatitis B and C, hepatitis E is mostly a mild infection, although it has a high mortality in pregnant women and can evolve to chronicity in immunocompromised patients. Ribavirin and pegylated interferon-α are the only available therapies, but both have side effects that are not acceptable for prophylaxis or treatment of mild infections. In addition, these drugs cannot be used for all patient types (e.g. in case of pregnancy, specific organ transplants or co-morbidities) and in resource-poor settings. Hence there is an urgent need for better antiviral treatments that are efficacious and safe, also during pregnancy. In this review, a concise introduction to the virus and disease is provided, followed by a discussion of the available assay systems and potential molecular targets (viral proteins and host factors) for the development of inhibitors of HEV replication. Finally, directions for future research are presented.

KEYWORDS

hepatitis E virus; antiviral therapy; inhibitor; host factor; ribavirin; interferon

1. INTRODUCTION

Hepatitis E virus (HEV) is a feco-orally transmitted pathogen and one of the most common causes of acute hepatitis worldwide. Although the virus was already identified in 1983 (Balayan et al., 1983), many of its epidemiological and clinical features are becoming clear just recently. HEV is responsible for both acute outbreaks in developing countries and sporadic cases worldwide and is more and more being recognized as a problem in the transplant setting (Kamar et al., 2008a; Pischke et al., 2012). Although most infections are self-resolving, hepatitis E is characterized by a high mortality in pregnant women and can lead to chronic infections in immunocompromised patients. A vaccine has recently been approved for the Chinese, but not for other markets (Zhu et al., 2010). Current therapeutic options (i.e. ribavirin and interferon-α) have severe side effects and treatment failure has been reported. It may be important to have potent and safe inhibitors of HEV replication at hand to rapidly contain outbreaks and to treat immunodeficient patients.

In this review, a general introduction to HEV is presented, followed by an overview of different potential antiviral targets and currently available methods for drug testing. Directions for developing antivirals against the HEV will be suggested.

2. HEPATITIS E VIRUS

a. Genome structure

HEV has a capped positive-sense single-stranded RNA genome that comprises a 5' untranslated region (UTR), open reading frames (ORF) 1 to 3 and a 3' UTR followed by a polyA-tract (see figure 1). ORF1 encodes the non-structural proteins and contains methyltransferase, protease, macrodomain, helicase and RNA-dependent RNA polymerase (RdRp)-encoding sequences (Koonin et al., 1992). Between the protease and the macrodomain, a hypervariable region containing a proline-rich hinge was found (Smith et al., 2012). The overlapping ORFs 2 and 3 are translated from a single subgenomic RNA into the structural proteins (Graff et al., 2006). ORF2 encodes the 660-aa capsid protein that consists of a shell domain (S), a middle domain (M, also called P1) and a protruding domain (P, also

called P2) (Guu et al., 2009; Yamashita et al., 2009). The protein translated from ORF3 is 114 aa in length, is phosphorylated (Zafrullah et al., 1997) and unique to HEV and closely related viruses.

b. Replication cycle

Although understudied, the HEV replication cycle seems typical for a single-stranded RNA virus of positive polarity (see figure 2). The virus particle first binds to heparan sulphate proteoglycans (HSPGs) on the host cell membrane (Kalia et al., 2009), transfers to its (unknown) cellular membrane receptor(s) and the resulting complex is internalized through clathrin-mediated endocytosis (Kapur et al., 2012). Consequently, the capped viral genome is released from the virion during the uncoating process and directly translated by the host cell ribosomal machinery. The non-structural proteins thus generated allow for the replication of the viral genome (probably in specific viral replication complexes) and the production of subgenomic RNA that is translated into the structural proteins from ORF2 and ORF3. Full-length RNA progeny is assembled with ORF2 capsid protein into viral particles of 27 to 30 nm (Balayan et al., 1983) that are consequently released from the cell in a non-lytic fashion. During release, HEV particles probably acquire a lipid bilayer (envelope) and associated ORF3 protein that are removed later by bile salts and enteric proteases respectively (Takahashi et al., 2010; Yamada et al., 2009).

c. Genetic diversity

HEV is classified into the *Hepevirus* genus in the family of the *Hepeviridae* (ref ICTV). At least 4 genotypes are currently recognized (Smith et al., 2013); genotypes 1 and 2 solely infect humans while genotypes 3 and 4 are zoonotic agents with their main reservoir in domestic pigs (Feagins et al., 2007; Meng et al., 1997; Tamada et al., 2004). Two additional genotypes have been proposed for HEV isolates from Japanese wild boars (Smith et al., 2013). Several viruses that are more or less related to HEV have been isolated from different species, including chickens (avian HEV, (Haqshenas et al., 2001)), rabbits (Zhao et al., 2009), rats (Johne et al., 2010), bats (Drexler et al., 2012), ferrets (Raj et al., 2012) and trout (Batts et al., 2011) (figure 3). The exact classification of these viruses

remains to be determined (Smith et al., 2013) and future discovery of additional hepeviruses seems likely.

3. HEPATITIS E

a. Epidemiology of disease

HEV genotypes 1 and 2 only infect humans and are endemic in many developing regions where they tend to cause large-scale water-borne outbreaks (Corwin et al., 1996; Naik et al., 1992). Genotype 1 is mainly found in Asia and Africa (Sugitani et al., 2009; Teshale et al., 2010), while genotype 2 has been isolated from Mexico and Africa, but seems less common (Maila et al., 2004; Velázquez et al., 1990) (see figure 4). It is estimated that infections with these genotypes result in 70,000 deaths annually (Rein et al., 2012). Genotypes 3 and 4 on the other hand are zoonotic agents with their main reservoir in domestic pigs (Colson et al., 2010; Li et al., 2005; Takahashi et al., 2004). Genotype 3 has a worldwide distribution, while genotype 4 is mostly found in Asia (Miyashita et al., 2012; Wang et al., 2012). However, genotype 4 has also been isolated from European pigs and patients recently (Hakze-van der Honing et al., 2011; Jeblaoui et al., 2013). Genotype 3 and 4 infections often result from consumption of undercooked pig or deer meat and may thus cause sporadic cases of hepatitis E. Although exact epidemiological data are missing, these infections seem a lot more common than initially thought (Dalton et al., 2008; Ijaz et al., 2009; Versluis et al., 2013).

b. Clinical syndromes

Acute hepatitis E is characterized by jaundice, fatigue, gastrointestinal discomfort etc. and is clinically indistinguishable from for instance hepatitis A (Khuroo, 1980), although many infections remain asymptomatic. Most cases of hepatitis E resolve spontaneously, but in some patients the infection may progress to fulminant hepatitis. Overall mortality rates are estimated between 0.5 and 4% (e.g. (Khuroo, 1980; Teshale et al., 2010)).

One particularly intriguing characteristic is that hepatitis E is often lethal in pregnant women with mortality rates of up to 20-25% (Khuroo et al., 1981; Tsega et al., 1993), although this has only been

observed for genotype 1 infections. Increased frequencies of obstetric complications and stillbirths have also been reported (Patra et al., 2007). The underlying mechanisms of this phenomenon (hormonal, immunological, genetical factors, etc.) are unclear to date.

A more recent finding is that HEV can establish chronic infections in immunocompromised subjects such as transplant and HIV-infected patients (Colson et al., 2009; Dalton et al., 2009; Kamar et al., 2008b). This has only been reported for genotype 3 thus far. Such chronic infections are rarely symptomatic and are sometimes mistaken for drug-induced liver injury (Davern et al., 2011). Chronic hepatitis E may lead to cirrhosis in 10% of patients (Kamar et al., 2011) and can ultimately result in death.

Occasional extrahepatic manifestations have also been reported; these are mostly neurological complications such as Guillain-Barré syndrome and neuralgic amyotrophy (Fong and Illahi, 2009; Kamar et al., 2010b; Sood et al., 2000) or nefrological manifestations such as glomerulonephritis (Kamar et al., 2012).

As evident from the discussion above, hepatitis E is profoundly different from the well-known disease courses of hepatitis B and C. While a considerable portion of patients infected with the hepatitis B virus (HBV) and especially the hepatitis C virus (HCV) evolves to chronicity, spontaneous recovery is the rule for hepatitis E where chronic disease has thus far only been reported in immunocompromised patients. The high rate of mortality in pregnant women is also unique to hepatitis E and no similar phenomena have been reported for hepatitis A, B or C.

c. Diagnostic methods

The most frequently used tests for diagnosing hepatitis E are detection of IgM and IgG antibodies by enzyme-linked immunosorbent assays (ELISA) and detection of viral RNA by (quantitative) reverse transcription PCR (RT-PCR). Presence of IgM antibodies indicates a recent infection, while IgG antibodies appear later and persist afterwards, at least for some time, indicating a past exposure. The current ELISAs suffer from large variability between assays (Bendall et al., 2010; Drobeniuc et al.,

2010; Mast et al., 1998) and therefore require urgent optimization and validation. Acute infection is usually confirmed by (quantitative) RT-PCR (e.g. (Jothikumar et al., 2006)), although these assays should be standardized as well to increase reliability.

Since the incubation and symptomatic periods for acute hepatitis E are only a few weeks, the successful use of antiviral drugs for acute cases of hepatitis E would depend on the availability of a rapid and inexpensive diagnostic test. Such test should also be easy to employ in resource-poor settings.

4. CURRENT CONTROL STRATEGIES

a. Vaccines

Vaccination would be an effective strategy to prevent HEV infection. Recently, such a HEV vaccine based on a recombinant truncated capsid protein was approved in China (Hecolin®, (Zhu et al., 2010)). It remains unclear whether the manufacturer intends to obtain market approval in other countries as well. Another recombinant vaccine successfully completed phase 2 clinical trials in Nepal (Shrestha et al., 2007), but further development was halted. Such vaccines may be useful as a prophylactic measure in high-risk patients such as immunocompromised patients and pregnant women. This would require specific clinical trials to demonstrate protective efficacy in these populations; to the best of our knowledge, such studies have not yet been reported for Hecolin®. However, the organization of large-scale vaccination campaigns against HEV may not be particularly cost-effective given the mostly benign course of infection in developing countries and the relatively low incidence in the developed world.

b. Other prophylactic measures

Since HEV is transmitted feco-orally, sanitary measures are probably the most effective strategy available to prevent viral spread. This is especially the case for genotypes 1 and 2 that often occur in large water-borne outbreaks. Availability of clean drinking water and proper disposal of waste water would be effective control strategies. Genotypes 3 and 4 are mostly spread through contaminated

157 food stuffs, such as the infamous French figatellu sausage made from uncooked pig liver (Berto et al.,
158 2013a; Colson et al., 2010). Thorough heating effectively kills the virus and prevents infection
159 (Feagins et al., 2008a). Although ineffectively, HEV can also be transmitted via blood transfusion
160 (Boxall et al., 2006; Colson et al., 2007), suggesting that screening of donor blood may be warranted.
161 In addition, vertical transmission of HEV has been reported (Khuroo et al., 1995), indicating that extra
162 caution is required in pregnant women.

163 **5. WHY DO WE NEED ANTIVIRAL DRUGS AGAINST HEPATITIS E VIRUS?**

164 **a. Current standard of care**

165 Chronic HEV infections in transplant patients can occasionally be resolved by decreasing
166 immunosuppresion, leading to spontaneous clearance in about 30% of the patients (Kamar et al.,
167 2011). When this approach is unsuccessful and for other types of patients, there are generally 2
168 possibilities: the use of pegylated interferon-alpha (PEG-IFNα) or ribavirin. PEG-IFNα has been used
169 successfully in liver transplant, kidney transplant and leukemia patients with chronic hepatitis E (Alric
170 et al., 2010; Haagsma et al., 2010; Kamar et al., 2010a, 2010c). However, ribavirin seems to be the
171 drug of choice in most cases with successful treatments being reported for multiple types of
172 transplant patients (Kamar et al., 2010d; Mallet et al., 2010; Pischke et al., 2013, 2012), but also for
173 HIV and leukemia patients with chronic hepatitis E (Giordani et al., 2013; Neukam et al., 2013). Acute
174 hepatitis E seems to respond to ribavirin treatment as well (Gerolami et al., 2011; Pischke et al.,
175 2013). In addition, the anti-HEV activity of both drugs has been confirmed *in vitro* (Debing et al.,
176 2013a). Nevertheless, they need to be administered for at least 3 months and are known for their
177 severe adverse effects. For PEG-IFNα for instance, this includes influenza-like symptoms and
178 neuropsychiatric side effects (Manns et al., 2006). Ribavirin on the other hand may induce severe
179 anemia necessitating dose reductions (Kamar et al., 2010d; Pischke et al., 2012). These reductions
180 may in turn lead to treatment failure (Pischke et al., 2013). One may deem such severe side effects
181 acceptable in light of the severity of chronic hepatitis E (as is the case for chronic hepatitis C as well),
182 but this is certainly not true when these drugs would be used for prophylaxis or to treat mild

infections. In addition, both ribavirin and PEG-IFNα are contra-indicated in pregnant women and other specific patient populations, leaving no available treatment options for these people. Also when considering acute infections during large outbreaks in developing countries, PEG-IFNα is not an option due to the fact that parenteral administration is needed and the required follow-up of hematological parameters during ribavirin treatment is difficult to implement in such settings.

b. Future therapy for hepatitis E

For the reasons stated above, safe and effective treatments for hepatitis E are urgently required. In case of acute infections, antiviral drugs would be useful to shorten the period of illness and prevent progression to fulminant hepatic failure. Such application would require rapid diagnosis of hepatitis E (see also section 3.c.). Another possibility would be to use antivirals to halt viral spread during epidemics and outbreaks. The fact that these outbreaks occur rather often in refugee camps (e.g. (Ahmed et al., 2013; Centers for Disease Control and Prevention (CDC), 2013)) may represent an opportunity for clinical trials testing the prophylactic potential of HEV inhibitors if such studies would be appropriate from an ethical point-of-view.

In pregnant women, such drugs could be life-saving for both mother and child, on the condition that they are devoid of teratogenic and significant adverse effects. Ideally, these women should be screened regularly with a rapid and sensitive HEV test and treated as soon as possible. This may be difficult to implement in developing countries where genotype 1 is endemic, so an approved HEV inhibitor should be sufficiently potent to arrest an established (fulminant) infection. Modulation of the hormonal system may be another interesting therapeutic option (see suggestions in section 7.f.), although the development of such strategy would require more detailed insights into disease pathogenesis.

For chronically infected patients, rapid clearance of HEV is required before irreversible progression to cirrhosis has occurred. Ideally, these drugs should be applicable in a diverse range of immunocompromised patients without overt toxicity. Since ribavirin treatment has a rather good

efficacy in chronically HEV-infected patients, a combination regimen with another HEV-inhibitor could be envisaged (as is currently being explored for HCV: ribavirin + direct-acting antiviral(s), e.g. (Osinusi et al., 2013; Zeuzem et al., 2013)). This may allow decreasing the ribavirin dosage, thus reducing and hopefully avoiding anemia and other side effects. In addition, we observed a slight synergistic effect *in vitro* for the combination of ribavirin and interferon-α (Debing et al., 2013a), suggesting that such a combination regimen may be worth considering in the clinical setting. Successful combination therapy has already been reported for a chronically HEV-infected HIV patient (Dalton et al., 2011b).

Developing antiviral drugs for the treatment of HEV infections will commercially not be as rewarding as for instance the development of HCV inhibitors (Debing et al., 2013b). Therefore, it appears opportune to consider other strategies than the classical discovery-development of completely new chemical entities as well, for instance off-label use of drugs with robust anti-HEV activity that are registered for unrelated indications when such molecules would be identified.

6. CURRENTLY AVAILABLE METHODS FOR DRUG TESTING

a. *In vitro* testing

Efficient cell culture systems for HEV have only recently become available. The Okamoto research group reported on both genotype 3 and 4 strains that replicate in PLC/PRF/5 (hepatoma) and/or A549 (lung adenocarcinoma) cell lines (Takahashi et al., 2010; Tanaka et al., 2009, 2007). The PLC/PRF/5 cells were also employed in a 3D cell culture system for HEV (Berto et al., 2013b). On the other hand, the NIH research group of Emerson derived a passage 6 virus of the Kernow-C1 strain that replicates in Huh7 and especially HepG2/C3A hepatoma cells (Shukla et al., 2012, 2011). This strain is derived from a chronically infected patient and contains an inserted human ribosomal S17 RNA fragment in the hypervariable region (Shukla et al., 2011). Detailed analyses indicated that this fragment and several additional mutations contributed to the improved *in vitro* replication. Although the replication capacity of this strain is sufficient for virus yield assays (e.g. (Debing et al., 2013a)),

overall growth kinetics are still rather slow. No cytopathic effect (CPE) has been described for any of these HEV strains, precluding the use of standard virology techniques such as CPE reduction assays and plaque assays. In addition, genotype 1 replicates only poorly in cell culture (Nguyen et al., 2013) while it probably accounts for most of the human infections worldwide.

Several replicon constructs have also been established in which the 5' part of the structural proteins is replaced by a GFP, neomycin resistance or luciferase gene (Graff et al., 2006; Shukla et al., 2012). The Kernow-C1 p6/luc reporter replicon proved especially useful in our hands as transient transfection into Huh7 cells allows for a 3-day antiviral assay to be performed (Debing et al., 2013a). For large-scale cell-based screenings with such replicons, a selectable reporter replicon would be highly convenient.

Another possible strategy to identify novel inhibitors of HEV replication is to use a surrogate virus. We recently reported that the cutthroat trout virus (CTV) may serve as such a surrogate (Debing et al., 2013c). CTV was isolated in 1988 from spawning trout in the western USA (Hedrick et al., 1991) and recent studies revealed that the virus belongs to its own genus in the *Hepeviridae* family (Smith et al., 2013). It has a remarkable similarity to HEV, especially when considering the helicase and polymerase sequences (Batts et al., 2011; Debing et al., 2013c). CTV replicates readily in cell culture allowing for RT-qPCR-based virus yield assays to be performed (Debing et al., 2013c). In addition, its *in vitro* replication kinetics can be further improved through repeated passaging (our unpublished results). In addition, CTV is a cytopathic virus. The CPE induced in CHSE-214 cells is rather limited, but we observed that nearly complete CPE can be attained in other piscine cell lines (our unpublished results). The avian HEV has also been successfully employed as a surrogate model (e.g. (Kenney et al., 2012; Pudupakam et al., 2011)).

In conclusion, recent developments have considerably expanded the number of assays and tools available for HEV antiviral studies. However, the possibilities are still rather limited and the field

would benefit from for instance HEV strains with improved replication kinetics, enzymatic assays for the different non-structural proteins and selectable reporter replicons.

b. Evaluation in laboratory animals

An ideal animal model for HEV would be a small inexpensive animal (preferably rodent) that can be infected with human HEV or at least a closely related virus and that develops acute hepatitis with a clear increase in liver markers and with high viral titers. Mortality is not required, although a lethal phenotype is highly useful for antiviral drug testing. An animal model that mimics the hormone-dependent effects observed in pregnant women would be a plus.

Several animal models are available for *in vivo* HEV studies (see table 1 for an overview), but they all have certain limitations. For instance, chicken and trout can be used as experimental animal models when infected with avian HEV and CTV respectively (Billam et al., 2005; Hedrick et al., 1994). Since both viruses are only surrogates for human HEV, further validation of experimental results would be required in another model. Genuine HEV infections can be performed in pigs ((Feagins et al., 2008b; Halbur et al., 2001; Sanford et al., 2011) and non-human primates such as macaques and chimpanzees (Emerson et al., 2001; Meng et al., 1998; Purcell et al., 2003; Yu et al., 2010). , However, ethical concerns as well as practical issues may make such studies complicated. Rodents may present a more accessible model organism. For instance, laboratory rats can be experimentally infected with rat HEV, but contradicting reports have been published for the human HEV genotypes (Li et al., 2013a, 2013b; Purcell et al., 2011; Zhu et al., 2013). Successful infection of Balb/c nude but not C57BL/6 mice with HEV was reported (Huang et al., 2009; Li et al., 2008), while rabbits can be infected with rabbit HEV and certain genotype 4 strains (Cheng et al., 2012). Finally, infection of Mongolian gerbils with genotype 4 has also been reported (Li et al., 2009). Due to the large variability between published results, a robust model that has been evaluated by multiple laboratories has not yet been reported. In addition, none of these models resulted in a lethal phenotype. For the predominant genotype 1 HEV, primates are even the only available model at this time. Promising

strategies towards a robust animal model may be infection of immunedeficient rats or mice with rat HEV, discovery studies for a murine HEV species, HEV infection of mice with chimeric human or porcine livers (Meuleman et al., 2005) or establishment of transgenic mice with a human HEV receptor (when identified), as was done successfully for poliovirus (Ren et al., 1990) and HCV (Dorner et al., 2011).

c. Clinical trials

When a HEV inhibitor would be shown to be successful in a (surrogate) animal model and would have an excellent safety and pharmacokinetic profile, clinical studies would be the next stage in development. For these studies, one could consider treating chronically infected patients, possibly in a combination regimen with ribavirin. However, these patients may have multiple co-morbidities and there could be confounding factors such as concomitant drug use leading to drug-drug interactions. Since hepatitis E has a rather long incubation period and clinical symptoms are often apparent for several weeks, clinical studies could possibly be performed in an outbreak setting as well; in this way, both prophylactic and therapeutic efficacy may be assessed.

7. POTENTIAL DRUG TARGETS

Below we present a selection of viral and host proteins may be attractive targets for antiviral therapy (see also table 2).

a. Inhibitors of HEV entry

The entry of a virus particle into the host cell is a multi-step process that involves attachment of the virus to its receptor on the cellular membrane, internalization of the virion-receptor complex and subsequent uncoating of the viral capsid releasing the RNA genome into the cytoplasm. The functional HEV receptor is still unknown to date, although there are indications that genotype 1 may use a different receptor(s) than genotype 3 (Nguyen et al., 2013). It was also found that HEV attaches to HSPGs, more specifically to 6-O-sulfated syndecan-1, which may account for its liver tropism (Kalia et al., 2009). It is hypothesized that HSPGs act as a highly abundant initial attachment receptor that

binds HEV with relatively low affinity. In a second step, the virion probably transfers to a high-affinity functional receptor and the resulting complex is internalized. Structure determination of a HEV-like particle combined with mutation and cell binding studies have identified a receptor binding site on top of the P2 dimer that protrudes from the 2-fold axis of symmetry (Yamashita et al., 2009). Interestingly, this site is homologous to the norovirus receptor-binding site that attaches to histo-blood group trisaccharides (Guu et al., 2009). In addition, another sialic acid-binding site was found in the P1 region of the capsid protein (Guu et al., 2009).

Despite the limited information that is available on HEV attachment and entry, several potentially interesting antiviral strategies can be derived from these data. First, the homology between the putative HEV and norovirus receptor-binding sites suggests that norovirus attachment inhibitors could possibly be interesting leads for further development into anti-HEV drugs. No confirmed norovirus entry inhibitors have been described to date, but two potentially interesting prototype molecules were designed through NMR binding studies (Rademacher et al., 2011) and in a similar way, citrate was found to compete with histo-blood group saccharides for norovirus binding (Hansman et al., 2012), suggesting a promising role for glycomimetics. A second and possibly overlapping approach is to search for inhibitors of HEV attachment to HSPGs. Heparan sulphate and related molecules inhibit the attachment of multiple viruses, both *in vitro* and *in vivo* (e.g. (Ali et al., 2012; Bugatti et al., 2007; Lee et al., 2006)). However, the clinical applicability of such molecules remains questionable and would require complete abolishment of any anticoagulative properties. Pentosan polysulphate is such an oligosaccharide derivative that is approved and marketed for pain relief in interstitial cystitis (Davis et al., 2008). Given that this drug inhibits entry of multiple viruses (Baba et al., 1988; García-Villalón and Gil-Fernández, 1991), it may be worthwhile to test it as a therapeutic option for hepatitis E, although it still has some anticoagulant properties (Scully et al., 1983). Thirdly, it has been shown that a truncated ORF2 protein is capable of preventing HEV infection *in vitro* (He et al., 2008). Through further truncation of this protein or otherwise mimicking the receptor-binding region of HEV, it would theoretically be possible to prevent virions from binding

to their cellular receptor. To illustrate the feasibility of such an approach, we refer to Myrcludex-B which is a lipopeptide derived from the HBV envelope protein and efficiently inhibits HBV entry *in vitro* and *in vivo* (Volz et al., 2013).

The strategies outlined above illustrate that the lacking of an identified receptor is an important hurdle for the identification and study of attachment, entry and uncoating inhibitors for HEV. Now that efficient cell culture systems are available (e.g. (Shukla et al., 2012)), this hiatus can and should be addressed.

b. RNA-dependent RNA polymerase inhibitors

Following release of the viral RNA in the cytoplasm and initial translation, the RNA genome is replicated by an RNA-dependent RNA polymerase. Sequence alignments predicted an RdRp-encoding sequence at the 3' end of ORF1 (see figure 1, (Koonin et al., 1992)) that belongs to the alphavirus-supergroup of RdRp's (Koonin, 1991). The polymerase activity was later confirmed by detection of newly synthesized negative-sense viral RNA *in vitro* (Agrawal et al., 2001; Rehman et al., 2008). The RdRp interacts with stem loops in the 3' end of the viral genome and with the polyA-tail (Agrawal et al., 2001) and co-localizes with the endoplasmatic reticulum (Rehman et al., 2008). However, further details about the HEV RdRp structure and function are missing.

As evidenced by the success of nucleoside and nucleotide inhibitors of HIV and herpesviruses, viral polymerases are attractive targets for antiviral therapy. Also the RdRp of RNA viruses has been targeted successfully by such molecules, consider for instance the nucleoside analogues that were developed against HCV, e.g. sofosbuvir is highly active, safe and has a high barrier to resistance. Sofosbuvir has successfully completed phase 3 clinical trials (Lawitz et al., 2013) and may receive market approval in the near future. 2'-C-methylcytidine (2'CMC) is a related molecule and was the first nucleoside analogue that entered clinical studies for the treatment of HCV infections. It has been shown to *in vitro* inhibit the replication of a number of other positive-sense single-stranded RNA viruses (Julander et al., 2010; Rocha-Pereira et al., 2013). However, neither 2'CMC nor a number of

its analogues exhibited significant activity in the transient HEV replicon assay (as described in (Debing et al., 2013a), unpublished results). Also only moderate antiviral activity of 2'CMC was observed against CTV (Debing et al., 2013c). Despite these negative results, other nucleos(t)ide analogues may possibly be interesting inhibitors of HEV replication. Moreover, research efforts could be directed towards developing an RdRp inhibitor that targets both HEV and the distantly related alphavirus family. Such a broad-spectrum inhibitor would provide a much-needed treatment option for multiple infections by neglected viruses such as the Chikungunya virus, Sindbis virus and the equine encephalitis viruses. Elucidation of the HEV RdRp crystal structure and development of a convenient enzymatic assay would allow to jumpstart this specific domain of HEV antiviral research.

c. Methyltransferase inhibitors

The HEV genome has a 5' cap structure which is a crucial requirement for infectivity (Emerson et al., 2001). Capping is a multistep process involving hydrolysis of the γ-phosphate from the terminal nucleotide, subsequent conjugation to GMP resulting in a 5'-ppp-5' triphosphate bond and finally methylation of the newly added guanosine. The 5' proportion of the ORF1 sequence was predicted to encode a viral methyltransferase (Koonin et al., 1992) and indeed, the expressed protein displays guanyltransferase and guanine-7-methyltransferase activities (Magden et al., 2001). The missing RNA triphosphatase function may be fulfilled by the HEV helicase (Karpe and Lole, 2010a).

Since HEV is heavily dependent on the capping of its RNA genome for infectivity, the viral methyltransferase seems an ideal target for antiviral therapy, all the more since these enzymatic activities are virus-specific (Magden et al., 2001) and thus may allow for development of selective inhibitors. Successful targeting of viral methyltransferases has been described for instance for dengue virus (Barral et al., 2013) and West Nile virus (Chen et al., 2013). Moreover, a highly efficient inhibitor of Chikungunya virus replication that selects for resistance mutations in nsP1 (viral methyltransferase) was recently identified in our laboratory (unpublished results; Delang L., Leyssen P. & Neyts J.); indicating that inhibition of this transferase may be a valuable strategy to inhibit the

383 replication of RNA viruses, including HEV. Solving the crystal structure and development of robust
384 enzymatic assays would be significant advances.

385 **d. Helicase inhibitors**

386 RNA viruses require the RNA-unwinding activity of a virally encoded helicase enzyme for replication;
387 the energy needed for this process is provided by the hydrolysis of NTPs (Kwong et al., 2005). As for
388 the RdRp and methyltransferase, the NTPase and RNA unwinding activities of the predicted HEV
389 helicase domain have been confirmed *in vitro* (Karpe and Lole, 2010b). This helicase belongs to the
390 5'-to-3' unwinding SF-1 helicase superfamily, which also comprises the alphavirus and coronavirus
391 (CoV) helicases. Potent inhibitors have been identified for the SARS-CoV nsP13 helicase (Adedeji et
392 al., 2012; Tanner et al., 2005), suggesting that it may also be possible to develop inhibitors of the HEV
393 helicase. An important caveat is that such inhibitors need to be highly specific for viral helicases
394 without affecting host helicase activity to avoid toxicity. Examples from the herpesvirus field
395 (helicase-primase inhibitors such as letermovir which has successfully finished phase 2 clinical studies
396 (Marschall et al., 2012)) show that such selectivity can be attained.

397 **e. Targeting other viral proteins**

398 Besides the above mentioned enzymes, the HEV genome encodes several other proteins of which the
399 function and significance have not been elucidated completely, like the macro domain, the viral
400 protease and the ORF3 protein. Given that more information would become available, they may
401 constitute suitable targets for antiviral therapy.

402 The macrodomain, formerly known as the X-domain, is located just upstream of the helicase gene
403 (figure 1, (Koonin et al., 1992)). Its name is derived from the homologous non-histone domain of the
404 macroH2A histone (Egloff et al., 2006). The exact function of the HEV macrodomain is unclear,
405 although it strongly binds poly(ADP-ribose) (Egloff et al., 2006), an important post-translational
406 protein modification involved in cell survival and apoptosis, among other functions (Schreiber et al.,
407 2006). Since it has been suggested that the biological function of viral macrodomains may be

408 unrelated to that of their human homologs (Neuvonen and Ahola, 2009), they could represent
409 attractive targets for inhibition. However, this would require a more profound understanding of the
410 function and activity of this domain.

411 Secondly, a papain-like cysteine protease domain has been predicted in the HEV genome sequence
412 (Koonin et al., 1992), but it remains unclear whether this protease is fully functional. Consequently,
413 one of the enigmas of HEV molecular biology is whether ORF1 is processed into discrete functional
414 units or not: several publications reported processing of ORF1 to some extent (Panda et al., 2000;
415 Parvez, 2013; Ropp et al., 2000; Sehgal et al., 2006), while others found no processing whatsoever
416 (Ansari et al., 2000; Perttilä et al., 2013; Suppiah et al., 2011). On the other hand, deubiquitination
417 activity has been reported for the methyltransferase-protease fusion domain which may be involved
418 in combating cellular antiviral responses (Karpe and Lole, 2011) and a recent mutational study
419 suggested a glycine-triad as the protease substrate sequence (Parvez, 2013). Although viral proteases
420 in general are interesting targets for antiviral therapy (as exemplified in the fields of HIV and HCV),
421 more insight into the activity, function and structure of the HEV protease would be essential before
422 focusing on protease inhibitors.

423 ORF3 encodes a 114aa phosphoprotein (Graff et al., 2006) that is exclusively found in hepeviruses.
424 ORF3 protein is dispensable for *in vitro* infection of cultured cells (Emerson et al., 2006), but required
425 for infection and seroconversion of rhesus macaques (Graff et al., 2005). A number of functions have
426 been proposed and several binding partners have been identified (Holla et al., 2013; Ratra et al.,
427 2008), but in general, ORF3 protein seems to optimize the host cell for HEV replication by increasing
428 cell survival (Chandra et al., 2010, 2008; Moin et al., 2007) and suppressing interferon-α signaling
429 (Dong et al., 2012). In addition, ORF3 protein has been implicated in virion egress (Yamada et al.,
430 2009) and disruption of blood coagulation (Geng et al., 2013). It should be noted that most of these
431 results have been obtained in artificial systems, i.e. overexpression of ORF3 protein in cultured cells
432 instead of genuine infection with HEV. In order to be targeted for antiviral development, more

insight into the ORF3 structure and function is required. Since the protein has multiple cellular binding partners, it may be interesting to look for inhibitors of these protein-protein interactions (see also section 7.f.). Such an approach may yield very specific inhibitors of HEV replication with low toxicity and a high barrier to resistance, especially given the overlap with ORF2 which is expected to substantially limit the number of viable mutations. The feasibility of identifying protein-protein interaction inhibitors as antiviral molecules was proven for the interaction between LEDGF/p75 and the HIV integrase for instance (Christ et al., 2010).

f. Host factors

Since many direct-acting antiviral agents are prone to the emergence and selection of resistant viruses, there is an increasing interest in targeting host factors that are co-opted by viruses for their own replication. Such host factor-targeting antivirals should have an increased barrier to resistance and may potentially have broad-spectrum activity. Multiple binding partners have been identified for HEV structural and non-structural proteins. The functional significance of many of these interactions remains unclear to date, but some of the host factors may be well-suited for antiviral targeting. Here, we discuss a few host factors that we believe have the most potential at being drugable.

First, tumor susceptibility gene 101 (Tsg101) is a central mediator in the endosomal protein sorting pathway (Surjit et al., 2006). HEV ORF3 protein has a PSAP-motif through which it associates with Tsg101, thus increasing secretion of the immunosuppressive α1-microglobulin and locally suppressing the host immune response (Surjit et al., 2006). In addition, Tsg101 is required for virion release by interacting with the ORF3 protein PSAP-motif (Kenney et al., 2012; Nagashima et al., 2011a, 2011b). The involvement of Tsg101 in virion release is not limited to HEV; it was also found to be essential for HIV and Ebola virus budding (Garrus et al., 2001; Martin-Serrano et al., 2001) and is being explored as a target for antiviral therapy (Chen et al., 2010). For instance, cyclic peptides blocking the interaction between Tsg101 and the PTAP motif of the HIV Gag-protein were found to block HIV budding (Tavassoli et al., 2008). Moreover, Tsg101 is expressed on the cell membrane

458 during both HIV and influenza virus infection and targeting by antibodies blocks virus production
459 (Bonavia et al., 2010; Diaz et al., 2010). Similar questions can be posed for HEV: does HEV induce
460 expression of Tsg101 at the cell membrane? Can the interaction between Tsg101 and ORF3 be
461 blocked with a small molecule or (cyclic) peptide? Potentially, antiviral research on HEV could piggy-
462 back on the ongoing efforts for HIV and influenza virus and thus advance rapidly.

463 Another interesting finding is that the proteasome inhibitor MG132 inhibits HEV replication,
464 indicating that HEV likely requires an active ubiquitin-proteasome system for infection (Karpe and
465 Meng, 2012). Similar findings have been reported for influenza virus (Haasbach et al., 2011), HIV
466 (Schubert et al., 2000) and vaccinia virus (Satheshkumar et al., 2009). This suggests that targeting the
467 proteasome system may be an interesting strategy for identifying broad-spectrum antiviral drugs.
468 Proteasome inhibitors such as bortezomib and carfilzomib are currently being used in the treatment
469 of multiple myeloma, based on the increased susceptibility of cancer cells compared to non-
470 malignant cells (Hideshima et al., 2001). However, it is unknown whether such a selectivity can also
471 be attained for virus-infected cells versus healthy cells to avoid severe side effects.

472 A similar analysis may apply to heat shock protein 90 (hsp90) as a cellular target for anti-HEV drugs
473 and antiviral therapy in general. Hsp90 is required for intracellular trafficking of HEV particles and
474 inhibition of hsp90 with geldanamycin blocks HEV infection (Zheng et al., 2010). Many viruses
475 depend on hsp90 and their replication can be blocked by hsp90 inhibitors (Basha et al., 2005; Geller
476 et al., 2013; Sun et al., 2013). Such inhibitors are currently being developed as anticancer therapies
477 ((Jhaveri and Modi, 2012) for an overview), but the question remains if sufficient selectivity can be
478 attained for safe antiviral therapy. An alternative strategy may be to specifically inhibit the protein-
479 protein interaction between the HEV capsid and hsp90, thus leaving hsp90 enzymatic activity
480 undisturbed.

481 Finally, there are multiple indications that HEV replication may be influenced by sex hormones. First,
482 mortality increases dramatically in pregnant women infected with genotype 1 (up to 25%, (Khuroo et

al., 1981; Tsega et al., 1993)). The underlying mechanism is still unclear, but it is known that low progesterone receptor mRNA levels and mutations in the progesterone receptor gene (PROGINS) are associated with poor pregnancy outcome (fulminant hepatic failure, fetal and maternal death) during HEV infection (Bose et al., 2011). On the other hand, autochthonous HEV infections in developed countries seem to primarily affect males over 50 (Dalton et al., 2011a; Davern et al., 2011; Mansuy et al., 2009). We reported that the replication of the CTV, a HEV surrogate virus, can be directly influenced by different concentrations of progesterone, testosterone and 17β-estradiol (Debing et al., 2013c), suggesting the possibility of a similar phenomenon in HEV infection. Several potential strategies can be derived from the limited information that is available. Since decreased progesterone receptor activity is associated with poor pregnancy outcome in hepatitis E (Bose et al., 2011) and high concentrations of progesterone inhibit CTV replication (Debing et al., 2013c), one may consider progesterone supplementation in pregnant women with hepatitis E. Such supplementation has been approved by the FDA for the prevention of preterm birth (Norwitz and Caughey, 2011). Similarly, testosterone levels decrease with age and low testosterone concentrations facilitate CTV replication (Debing et al., 2013c), suggesting that testosterone supplementation may be beneficial in elderly man (chronically) infected with HEV. A third possibility may be the use of selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene. SERMs were found to inhibit HCV replication *in vitro* (Murakami et al., 2013; Watashi et al., 2007) and raloxifene improved the efficacy of pegylated interferon + ribavirin in menopausal women chronically infected with HCV (Furusyo et al., 2012). It would be interesting to look into the effects of SERMs on HEV or CTV replication as they may represent another possible treatment option for hepatitis E (but not during pregnancy). To fully explore the potential treatment of hepatitis E through modulation of the hormonal system, a robust animal model displaying hormone-dependent virus replication would be much appreciated.

8. DIRECTIONS FOR FUTURE RESEARCH

Antiviral drugs for the treatment of hepatitis E are required for both acute and chronic infections. Such drugs should have an increased potency, efficacy and safety compared to the currently used

ribavirin and PEG-IFNα, thus allowing for prophylactic use, containment of outbreaks and preferably treatment of pregnant women and other high-risk patients as well. It would also be interesting to further characterize the mechanism of action of ribavirin and PEG-IFNα in hepatitis E. As evident from the discussion above, HEV is an understudied pathogen and most of the suggested antiviral strategies require a more elaborate fundamental knowledge of the molecular virology. In addition, better model systems should be developed, both *in vitro* and *in vivo*, for proper antiviral research to be conducted.

HEV is an extremely interesting pathogen that deserves more profound study. In the last decade, much effort has gone to the HCV antiviral field. As many (candidate) drugs are currently in advanced clinical development, awaiting market approval or even on the market, HEV research could potentially benefit from research groups switching their main focus from HCV to HEV.

ACKNOWLEDGMENTS

Yannick Debing is a fellow of the Research Fund – Flanders (FWO). The original work of the authors is also funded by EU FP7 project SILVER (260644).

REFERENCES

Adedeji, A.O., Singh, K., Calcaterra, N.E., DeDiego, M.L., Enjuanes, L., Weiss, S., Sarafianos, S.G., 2012. Severe acute respiratory syndrome coronavirus replication inhibitor that interferes with the nucleic acid unwinding of the viral helicase. Antimicrob. Agents Chemother. 56, 4718–4728.

Agrawal, S., Gupta, D., Panda, S.K., 2001. The 3' end of hepatitis E virus (HEV) genome binds specifically to the viral RNA-dependent RNA polymerase (RdRp). Virology 282, 87–101.

Ahmed, J.A., Moturi, E., Spiegel, P., Schilperoord, M., Burton, W., Kassim, N.H., Mohamed, A., Ochieng, M., Nderitu, L., Navarro-Colorado, C., Burke, H., Cookson, S., Handzel, T., Waiboci, L.W., Montgomery, J.M., Teshale, E., Marano, N., 2013. Hepatitis E outbreak, Dadaab refugee camp, Kenya, 2012. Emerg. Infect. Dis. 19, 1010–1012.

533 Ali, M.M., Karasneh, G.A., Jarding, M.J., Tiwari, V., Shukla, D., 2012. A 3-O-sulfated heparan sulfate
534 binding peptide preferentially targets herpes simplex virus 2-infected cells. J. Virol. 86, 6434–
535 6443.
536 Alric, L., Bonnet, D., Laurent, G., Kamar, N., Izopet, J., 2010. Chronic hepatitis E virus infection:
537 successful virologic response to pegylated interferon-alpha therapy. Ann. Intern. Med. 153,
538 135–136.
539 Ansari, I.H., Nanda, S.K., Durgapal, H., Agrawal, S., Mohanty, S.K., Gupta, D., Jameel, S., Panda, S.K.,
540 2000. Cloning, sequencing, and expression of the hepatitis E virus (HEV) nonstructural open
541 reading frame 1 (ORF1). J. Med. Virol. 60, 275–283.
542 Baba, M., Snoeck, R., Pauwels, R., de Clercq, E., 1988. Sulfated polysaccharides are potent and
543 selective inhibitors of various enveloped viruses, including herpes simplex virus,
544 cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. Antimicrob.
545 Agents Chemother. 32, 1742–1745.
546 Balayan, M.S., Andjaparidze, A.G., Savinskaya, S.S., Ketiladze, E.S., Braginsky, D.M., Savinov, A.P.,
547 Poleschuk, V.F., 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-
548 oral route. Intervirology 20, 23–31.
549 Barral, K., Sallamand, C., Petzold, C., Coutard, B., Collet, A., Thillier, Y., Zimmermann, J., Vasseur, J.-J.,
550 Canard, B., Rohayem, J., Debart, F., Decroly, E., 2013. Development of specific dengue virus
551 2'-O- and N7-methyltransferase assays for antiviral drug screening. Antiviral Res. 99, 292–
552 300.
553 Basha, W., Kitagawa, R., Uhara, M., Imazu, H., Uechi, K., Tanaka, J., 2005. Geldanamycin, a potent and
554 specific inhibitor of Hsp90, inhibits gene expression and replication of human
555 cytomegalovirus. Antivir. Chem. Chemother. 16, 135–146.
556 Batts, W., Yun, S., Hedrick, R., Winton, J., 2011. A novel member of the family Hepeviridae from
557 cutthroat trout (Oncorhynchus clarkii). Virus Res. 158, 116–123.

558 Bendall, R., Ellis, V., Ijaz, S., Ali, R., Dalton, H., 2010. A comparison of two commercially available anti-
559 HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries.
560 J. Med. Virol. 82, 799–805.
561 Berto, A., Grierson, S., Hakze-van der Honing, R., Martelli, F., Johne, R., Reetz, J., Ulrich, R.G., Pavio,
562 N., Van der Poel, W.H.M., Banks, M., 2013a. Hepatitis E virus in pork liver sausage, France.
563 Emerg. Infect. Dis. 19, 264–266.
564 Berto, A., Van der Poel, W.H.M., Hakze-van der Honing, R., Martelli, F., La Ragione, R.M., Inglese, N.,
565 Collins, J., Grierson, S., Johne, R., Reetz, J., Dastjerdi, A., Banks, M., 2013b. Replication of
566 hepatitis E virus in three-dimensional cell culture. J. Virol. Methods 187, 327–332.
567 Billam, P., Huang, F.F., Sun, Z.F., Pierson, F.W., Duncan, R.B., Elvinger, F., Guenette, D.K., Toth, T.E.,
568 Meng, X.J., 2005. Systematic pathogenesis and replication of avian hepatitis E virus in
569 specific-pathogen-free adult chickens. J. Virol. 79, 3429–3437.
570 Bonavia, A., Diaz, L.S., Santos, D., Cassella, J., Fesseha, Z., Bamba, D., Sui, B., Li, W.-B., Duan, R., Chen,
571 L.-M., Donis, R.O., Goldblatt, M., Kinch, M.S., 2010. Antibody targeting of TSG101 on
572 influenza-infected cells. Virus Adapt. Treat. 2010, 147–157.
573 Bose, P.D., Das, B.C., Kumar, A., Gondal, R., Kumar, D., Kar, P., 2011. High viral load and deregulation
574 of the progesterone receptor signaling pathway: association with hepatitis E-related poor
575 pregnancy outcome. J. Hepatol. 54, 1107–1113.
576 Boxall, E., Herborn, A., Kochethu, G., Pratt, G., Adams, D., Ijaz, S., Teo, C.-G., 2006. Transfusion-
577 transmitted hepatitis E in a “nonhyperendemic” country. Transfus. Med. Oxf. Engl. 16, 79–83.
578 Bugatti, A., Urbinati, C., Ravelli, C., De Clercq, E., Liekens, S., Rusnati, M., 2007. Heparin-mimicking
579 sulfonic acid polymers as multitarget inhibitors of human immunodeficiency virus type 1 Tat
580 and gp120 proteins. Antimicrob. Agents Chemother. 51, 2337–2345.
581 Centers for Disease Control and Prevention (CDC), 2013. Investigation of hepatitis E outbreak among
582 refugees - Upper Nile, South Sudan, 2012-2013. MMWR Morb. Mortal. Wkly. Rep. 62, 581–
583 586.

584 Chandra, V., Kalia, M., Hajela, K., Jameel, S., 2010. The ORF3 protein of hepatitis E virus delays
585 degradation of activated growth factor receptors by interacting with CIN85 and blocking
586 formation of the Cbl-CIN85 complex. J. Virol. 84, 3857–3867.
587 Chandra, V., Kar-Roy, A., Kumari, S., Mayor, S., Jameel, S., 2008. The hepatitis E virus ORF3 protein
588 modulates epidermal growth factor receptor trafficking, STAT3 translocation, and the acute-
589 phase response. J. Virol. 82, 7100–7110.
590 Chen, H., Liu, L., Jones, S.A., Banavali, N., Kass, J., Li, Z., Zhang, J., Kramer, L.D., Ghosh, A.K., Li, H.,
591 2013. Selective inhibition of the West Nile virus methyltransferase by nucleoside analogs.
592 Antiviral Res. 97, 232–239.
593 Chen, H., Liu, X., Li, Z., Zhan, P., De Clercq, E., 2010. TSG101: a novel anti-HIV-1 drug target. Curr.
594 Med. Chem. 17, 750–758.
595 Cheng, X., Wang, S., Dai, X., Shi, C., Wen, Y., Zhu, M., Zhan, S., Meng, J., 2012. Rabbit as a novel
596 animal model for hepatitis E virus infection and vaccine evaluation. PloS One 7, e51616.
597 Christ, F., Voet, A., Marchand, A., Nicolet, S., Desimmie, B.A., Marchand, D., Bardiot, D., Van der
598 Veken, N.J., Van Remoortel, B., Strelkov, S.V., De Maeyer, M., Chaltin, P., Debyser, Z., 2010.
599 Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV
600 replication. Nat. Chem. Biol. 6, 442–448.
601 Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., Heyries, L., Raoult, D.,
602 Gerolami, R., 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans.
603 J. Infect. Dis. 202, 825–834.
604 Colson, P., Coze, C., Gallian, P., Henry, M., De Micco, P., Tamalet, C., 2007. Transfusion-associated
605 hepatitis E, France. Emerg. Infect. Dis. 13, 648–649.
606 Colson, P., Kaba, M., Moreau, J., Brouqui, P., 2009. Hepatitis E in an HIV-infected patient. J. Clin. Virol.
607 Off. Publ. Pan Am. Soc. Clin. Virol. 45, 269–271.
608 Corwin, A.L., Khiem, H.B., Clayson, E.T., Pham, K.S., Vo, T.T., Vu, T.Y., Cao, T.T., Vaughn, D., Merven,
609 J., Richie, T.L., Putri, M.P., He, J., Graham, R., Wignall, F.S., Hyams, K.C., 1996. A waterborne

610 outbreak of hepatitis E virus transmission in southwestern Vietnam. Am. J. Trop. Med. Hyg.
611 54, 559–562.
612 Dalton, H.R., Bendall, R., Ijaz, S., Banks, M., 2008. Hepatitis E: an emerging infection in developed
613 countries. Lancet Infect. Dis. 8, 698–709.
614 Dalton, H.R., Bendall, R.P., Keane, F.E., Tedder, R.S., Ijaz, S., 2009. Persistent carriage of hepatitis E
615 virus in patients with HIV infection. N. Engl. J. Med. 361, 1025–1027.
616 Dalton, H.R., Bendall, R.P., Rashid, M., Ellis, V., Ali, R., Ramnarace, R., Stableforth, W., Headdon, W.,
617 Abbott, R., McLaughlin, C., Froment, E., Hall, K.J., Michell, N.P., Thatcher, P., Henley, W.E.,
618 2011a. Host risk factors and autochthonous hepatitis E infection. Eur. J. Gastroenterol.
619 Hepatol. 23, 1200–1205.
620 Dalton, H.R., Keane, F.E., Bendall, R., Mathew, J., Ijaz, S., 2011b. Treatment of chronic hepatitis E in a
621 patient with HIV infection. Ann. Intern. Med. 155, 479–480.
622 Davern, T.J., Chalasani, N., Fontana, R.J., Hayashi, P.H., Protiva, P., Kleiner, D.E., Engle, R.E., Nguyen,
623 H., Emerson, S.U., Purcell, R.H., Tillmann, H.L., Gu, J., Serrano, J., Hoofnagle, J.H., Drug-
624 Induced Liver Injury Network (DILIN), 2011. Acute hepatitis E infection accounts for some
625 cases of suspected drug-induced liver injury. Gastroenterology 141, 1665–1672.e1–9.
626 Davis, E.L., El Khoudary, S.R., Talbott, E.O., Davis, J., Regan, L.J., 2008. Safety and efficacy of the use of
627 intravesical and oral pentosan polysulfate sodium for interstitial cystitis: a randomized
628 double-blind clinical trial. J. Urol. 179, 177–185.
629 Debing, Y., Emerson, S.U., Wang, Y., Pan, Q., Balzarini, J., Dallmeier, K., Neyts, J., 2013a. Ribavirin
630 inhibits in vitro hepatitis E virus replication through depletion of cellular GTP pools and is
631 moderately synergistic with interferon-alpha. Antimicrob. Agents Chemother. In press.
632 Debing, Y., Jochmans, D., Neyts, J., 2013b. Intervention strategies for emerging viruses: use of
633 antivirals. Curr. Opin. Virol. 3, 217–224.

634 Debing, Y., Winton, J., Neyts, J., Dallmeier, K., 2013c. Cutthroat trout virus as a surrogate in vitro
635 infection model for testing inhibitors of hepatitis E virus replication. Antiviral Res. 100, 98–
636 101.
637 Diaz, L., Mao, H., Zhou, Y., Kohli, M., Cassella, J., Santos, D., Fesseha, Z., Weng, K., Chen, H., Bamba,
638 D., Marks, J.D., Goldblatt, M., Kinch, M., 2010. TSG101 exposure on the surface of HIV-1
639 infected cells: implications for monoclonal antibody therapy for HIV/AIDS. Am. J. Transl. Res.
640 2, 368–380.
641 Dong, C., Zafrullah, M., Mixson-Hayden, T., Dai, X., Liang, J., Meng, J., Kamili, S., 2012. Suppression of
642 interferon-α signaling by hepatitis E virus. Hepatol. Baltim. Md 55, 1324–1332.
643 Dorner, M., Horwitz, J.A., Robbins, J.B., Barry, W.T., Feng, Q., Mu, K., Jones, C.T., Schoggins, J.W.,
644 Catanese, M.T., Burton, D.R., Law, M., Rice, C.M., Ploss, A., 2011. A genetically humanized
645 mouse model for hepatitis C virus infection. Nature 474, 208–211.
646 Drexler, J.F., Seelen, A., Corman, V.M., Fumie Tateno, A., Cottontail, V., Melim Zerbinati, R., Gloza-
647 Rausch, F., Klose, S.M., Adu-Sarkodie, Y., Oppong, S.K., Kalko, E.K.V., Osterman, A., Rasche,
648 A., Adam, A., Müller, M.A., Ulrich, R.G., Leroy, E.M., Lukashev, A.N., Drosten, C., 2012. Bats
649 worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the
650 family Hepeviridae. J. Virol. 86, 9134–9147.
651 Drobeniuc, J., Meng, J., Reuter, G., Greene-Montfort, T., Khudyakova, N., Dimitrova, Z., Kamili, S.,
652 Teo, C.-G., 2010. Serologic assays specific to immunoglobulin M antibodies against hepatitis E
653 virus: pangenotypic evaluation of performances. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc.
654 Am. 51, e24–27.
655 Egloff, M.-P., Malet, H., Putics, A., Heinonen, M., Dutartre, H., Frangeul, A., Gruez, A., Campanacci, V.,
656 Cambillau, C., Ziebuhr, J., Ahola, T., Canard, B., 2006. Structural and functional basis for ADP-
657 ribose and poly(ADP-ribose) binding by viral macro domains. J. Virol. 80, 8493–8502.

658 Emerson, S.U., Nguyen, H., Torian, U., Purcell, R.H., 2006. ORF3 protein of hepatitis E virus is not
659 required for replication, virion assembly, or infection of hepatoma cells in vitro. J. Virol. 80,
660 10457–10464.
661 Emerson, S.U., Zhang, M., Meng, X.J., Nguyen, H., St Claire, M., Govindarajan, S., Huang, Y.K., Purcell,
662 R.H., 2001. Recombinant hepatitis E virus genomes infectious for primates: importance of
663 capping and discovery of a cis-reactive element. Proc. Natl. Acad. Sci. U. S. A. 98, 15270–
664 15275.
665 Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.J., 2008a. Inactivation of infectious
666 hepatitis E virus present in commercial pig livers sold in local grocery stores in the United
667 States. Int. J. Food Microbiol. 123, 32–37.
668 Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.-J., 2007. Detection and
669 characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery
670 stores in the USA. J. Gen. Virol. 88, 912–917.
671 Feagins, A.R., Opriessnig, T., Huang, Y.W., Halbur, P.G., Meng, X.J., 2008b. Cross-species infection of
672 specific-pathogen-free pigs by a genotype 4 strain of human hepatitis E virus. J. Med. Virol.
673 80, 1379–1386.
674 Fong, F., Illahi, M., 2009. Neuralgic amyotrophy associated with hepatitis E virus. Clin. Neurol.
675 Neurosurg. 111, 193–195.
676 Furusyo, N., Ogawa, E., Sudoh, M., Murata, M., Ihara, T., Hayashi, T., Ikezaki, H., Hiramine, S., Mukae,
677 H., Toyoda, K., Taniai, H., Okada, K., Kainuma, M., Kajiwara, E., Hayashi, J., 2012. Raloxifene
678 hydrochloride is an adjuvant antiviral treatment of postmenopausal women with chronic
679 hepatitis C: a randomized trial. J. Hepatol. 57, 1186–1192.
680 García-Villalón, D., Gil-Fernández, C., 1991. Antiviral activity of sulfated polysaccharides against
681 African swine fever virus. Antiviral Res. 15, 139–148.

682 Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein,
683 D.A., Stray, K.M., Côté, M., Rich, R.L., Myszka, D.G., Sundquist, W.I., 2001. Tsg101 and the
684 vacuolar protein sorting pathway are essential for HIV-1 budding. Cell 107, 55–65.
685 Geller, R., Andino, R., Frydman, J., 2013. Hsp90 inhibitors exhibit resistance-free antiviral activity
686 against respiratory syncytial virus. PloS One 8, e56762.
687 Geng, Y., Yang, J., Huang, W., Harrison, T.J., Zhou, Y., Wen, Z., Wang, Y., 2013. Virus host protein
688 interaction network analysis reveals that the HEV ORF3 protein may interrupt the blood
689 coagulation process. PloS One 8, e56320.
690 Gerolami, R., Borentain, P., Raissouni, F., Motte, A., Solas, C., Colson, P., 2011. Treatment of severe
691 acute hepatitis E by ribavirin. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 52, 60–62.
692 Giordani, M.T., Fabris, P., Brunetti, E., Goblirsch, S., Romanò, L., 2013. Hepatitis e and lymphocytic
693 leukemia in man, Italy. Emerg. Infect. Dis. 19, 2054–2056.
694 Graff, J., Nguyen, H., Yu, C., Elkins, W.R., St Claire, M., Purcell, R.H., Emerson, S.U., 2005. The open
695 reading frame 3 gene of hepatitis E virus contains a cis-reactive element and encodes a
696 protein required for infection of macaques. J. Virol. 79, 6680–6689.
697 Graff, J., Torian, U., Nguyen, H., Emerson, S.U., 2006. A bicistronic subgenomic mRNA encodes both
698 the ORF2 and ORF3 proteins of hepatitis E virus. J. Virol. 80, 5919–5926.
699 Guu, T.S.Y., Liu, Z., Ye, Q., Mata, D.A., Li, K., Yin, C., Zhang, J., Tao, Y.J., 2009. Structure of the hepatitis
700 E virus-like particle suggests mechanisms for virus assembly and receptor binding. Proc. Natl.
701 Acad. Sci. U. S. A. 106, 12992–12997.
702 Haagsma, E.B., Riezebos-Brilman, A., van den Berg, A.P., Porte, R.J., Niesters, H.G.M., 2010.
703 Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon
704 alpha-2b. Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc. 16,
705 474–477.

706 Haasbach, E., Pauli, E.-K., Spranger, R., Mitzner, D., Schubert, U., Kircheis, R., Planz, O., 2011. Antiviral
707 activity of the proteasome inhibitor VL-01 against influenza A viruses. Antiviral Res. 91, 304–
708 313.
709 Hakze-van der Honing, R.W., van Coillie, E., Antonis, A.F.G., van der Poel, W.H.M., 2011. First
710 isolation of hepatitis E virus genotype 4 in Europe through swine surveillance in the
711 Netherlands and Belgium. PloS One 6, e22673.
712 Halbur, P.G., Kasorndorkbua, C., Gilbert, C., Guenette, D., Potters, M.B., Purcell, R.H., Emerson, S.U.,
713 Toth, T.E., Meng, X.J., 2001. Comparative pathogenesis of infection of pigs with hepatitis E
714 viruses recovered from a pig and a human. J. Clin. Microbiol. 39, 918–923.
715 Hansman, G.S., Shahzad-Ul-Hussan, S., McLellan, J.S., Chuang, G.-Y., Georgiev, I., Shimoike, T.,
716 Katayama, K., Bewley, C.A., Kwong, P.D., 2012. Structural basis for norovirus inhibition and
717 fucose mimicry by citrate. J. Virol. 86, 284–292.
718 Haqshenas, G., Shivaprasad, H.L., Woolcock, P.R., Read, D.H., Meng, X.J., 2001. Genetic identification
719 and characterization of a novel virus related to human hepatitis E virus from chickens with
720 hepatitis-splenomegaly syndrome in the United States. J. Gen. Virol. 82, 2449–2462.
721 He, S., Miao, J., Zheng, Z., Wu, T., Xie, M., Tang, M., Zhang, J., Ng, M.-H., Xia, N., 2008. Putative
722 receptor-binding sites of hepatitis E virus. J. Gen. Virol. 89, 245–249.
723 Hedrick, R.P., LaPatra, S.E., Yun, S., Lauda, K.A., Jones, G.R., Congleton, J.L., de Kinkelin, P., 1994.
724 Induction of protection from infectious hematopoietic necrosis virus in rainbow trout
725 Oncorhynchus mykiss by pre-exposure to the avirulent cutthroat trout virus (CTV). Dis.
726 Aquat. Organ. 20, 111–118.
727 Hedrick, R.P., Yun, S., Wingfield, W.H., 1991. A Small RNA Virus Isolated from Salmonid Fishes in
728 California, USA. Can. J. Fish. Aquat. Sci. 48, 99–104.
729 Hideshima, T., Richardson, P., Chauhan, D., Palombella, V.J., Elliott, P.J., Adams, J., Anderson, K.C.,
730 2001. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes
731 drug resistance in human multiple myeloma cells. Cancer Res. 61, 3071–3076.

732 Holla, R.P., Ahmad, I., Ahmad, Z., Jameel, S., 2013. Molecular virology of hepatitis E virus. Semin.
733 Liver Dis. 33, 3–14.
734 Huang, F., Zhang, W., Gong, G., Yuan, C., Yan, Y., Yang, S., Cui, L., Zhu, J., Yang, Z., Hua, X., 2009.
735 Experimental infection of Balb/c nude mice with Hepatitis E virus. BMC Infect. Dis. 9, 93.
736 Ijaz, S., Vyse, A.J., Morgan, D., Pebody, R.G., Tedder, R.S., Brown, D., 2009. Indigenous hepatitis E
737 virus infection in England: more common than it seems. J. Clin. Virol. Off. Publ. Pan Am. Soc.
738 Clin. Virol. 44, 272–276.
739 Jeblaoui, A., Haim-Boukobza, S., Marchadier, E., Mokhtari, C., Roque-Afonso, A.-M., 2013. Genotype
740 4 hepatitis e virus in france: an autochthonous infection with a more severe presentation.
741 Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 57, e122–126.
742 Jhaveri, K., Modi, S., 2012. HSP90 inhibitors for cancer therapy and overcoming drug resistance. Adv.
743 Pharmacol. San Diego Calif 65, 471–517.
744 Johne, R., Heckel, G., Plenge-Bönig, A., Kindler, E., Maresch, C., Reetz, J., Schielke, A., Ulrich, R.G.,
745 2010. Novel hepatitis E virus genotype in Norway rats, Germany. Emerg. Infect. Dis. 16,
746 1452–1455.
747 Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J., Hill, V.R., 2006. A broadly reactive one-
748 step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J. Virol.
749 Methods 131, 65–71.
750 Julander, J.G., Jha, A.K., Choi, J.-A., Jung, K.-H., Smee, D.F., Morrey, J.D., Chu, C.K., 2010. Efficacy of 2'-
751 C-methylcytidine against yellow fever virus in cell culture and in a hamster model. Antiviral
752 Res. 86, 261–267.
753 Kalia, M., Chandra, V., Rahman, S.A., Sehgal, D., Jameel, S., 2009. Heparan sulfate proteoglycans are
754 required for cellular binding of the hepatitis E virus ORF2 capsid protein and for viral
755 infection. J. Virol. 83, 12714–12724.
756 Kamar, N., Abravanel, F., Garrouste, C., Cardeau-Desangles, I., Mansuy, J.M., Weclawiak, H., Izopet, J.,
757 Rostaing, L., 2010a. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis

E virus infection in a haemodialysis patient. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. 25, 2792–2795.

Kamar, N., Garrouste, C., Haagsma, E.B., Garrigue, V., Pischke, S., Chauvet, C., Dumortier, J., Cannesson, A., Cassuto-Viguier, E., Thervet, E., Conti, F., Lebray, P., Dalton, H.R., Santella, R., Kanaan, N., Essig, M., Mousson, C., Radenne, S., Roque-Afonso, A.M., Izopet, J., Rostaing, L., 2011. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. Gastroenterology 140, 1481–1489.

Kamar, N., Izopet, J., Cintas, P., Garrouste, C., Uro-Coste, E., Cointault, O., Rostaing, L., 2010b. Hepatitis E virus-induced neurological symptoms in a kidney-transplant patient with chronic hepatitis. Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg. 10, 1321–1324.

Kamar, N., Mansuy, J.-M., Cointault, O., Selves, J., Abravanel, F., Danjoux, M., Otal, P., Esposito, L., Durand, D., Izopet, J., Rostaing, L., 2008a. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg. 8, 1744–1748.

Kamar, N., Rostaing, L., Abravanel, F., Garrouste, C., Esposito, L., Cardeau-Desangles, I., Mansuy, J.M., Selves, J., Peron, J.M., Otal, P., Muscari, F., Izopet, J., 2010c. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 50, e30–33.

Kamar, N., Rostaing, L., Abravanel, F., Garrouste, C., Lhomme, S., Esposito, L., Basse, G., Cointault, O., Ribes, D., Nogier, M.B., Alric, L., Peron, J.M., Izopet, J., 2010d. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis e virus infection. Gastroenterology 139, 1612–1618.

Kamar, N., Selves, J., Mansuy, J.-M., Ouezzani, L., Péron, J.-M., Guitard, J., Cointault, O., Esposito, L., Abravanel, F., Danjoux, M., Durand, D., Vinel, J.-P., Izopet, J., Rostaing, L., 2008b. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N. Engl. J. Med. 358, 811–817.

784 Kamar, N., Weclawiak, H., Guilbeau-Frugier, C., Legrand-Abravanel, F., Cointault, O., Ribes, D.,
785 Esposito, L., Cardeau-Desangles, I., Guitard, J., Sallusto, F., Muscari, F., Peron, J.M., Alric, L.,
786 Izopet, J., Rostaing, L., 2012. Hepatitis E virus and the kidney in solid-organ transplant
787 patients. Transplantation 93, 617–623.
788 Kapur, N., Thakral, D., Durgapal, H., Panda, S.K., 2012. Hepatitis E virus enters liver cells through
789 receptor-dependent clathrin-mediated endocytosis. J. Viral Hepat. 19, 436–448.
790 Karpe, Y.A., Lole, K.S., 2010a. RNA 5'-triphosphatase activity of the hepatitis E virus helicase domain.
791 J. Virol. 84, 9637–9641.
792 Karpe, Y.A., Lole, K.S., 2010b. NTPase and 5' to 3' RNA duplex-unwinding activities of the hepatitis E
793 virus helicase domain. J. Virol. 84, 3595–3602.
794 Karpe, Y.A., Lole, K.S., 2011. Deubiquitination activity associated with hepatitis E virus putative
795 papain-like cysteine protease. J. Gen. Virol. 92, 2088–2092.
796 Karpe, Y.A., Meng, X.-J., 2012. Hepatitis E virus replication requires an active ubiquitin-proteasome
797 system. J. Virol. 86, 5948–5952.
798 Kenney, S.P., Pudupakam, R.S., Huang, Y.-W., Pierson, F.W., LeRoith, T., Meng, X.-J., 2012. The PSAP
799 motif within the ORF3 protein of an avian strain of the hepatitis E virus is not critical for viral
800 infectivity in vivo but plays a role in virus release. J. Virol. 86, 5637–5646.
801 Khuroo, M.S., 1980. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human
802 hepatitis virus distinct from post-transfusion non-A, non-B type. Am. J. Med. 68, 818–824.
803 Khuroo, M.S., Kamili, S., Jameel, S., 1995. Vertical transmission of hepatitis E virus. Lancet 345, 1025–
804 1026.
805 Khuroo, M.S., Teli, M.R., Skidmore, S., Sofi, M.A., Khuroo, M.I., 1981. Incidence and severity of viral
806 hepatitis in pregnancy. Am. J. Med. 70, 252–255.
807 Koonin, E.V., 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA
808 viruses. J. Gen. Virol. 72 (Pt 9), 2197–2206.

809 Koonin, E.V., Gorbalenya, A.E., Purdy, M.A., Rozanov, M.N., Reyes, G.R., Bradley, D.W., 1992.
810 Computer-assisted assignment of functional domains in the nonstructural polyprotein of
811 hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal
812 viruses. Proc. Natl. Acad. Sci. U. S. A. 89, 8259–8263.
813 Kwong, A.D., Rao, B.G., Jeang, K.-T., 2005. Viral and cellular RNA helicases as antiviral targets. Nat.
814 Rev. Drug Discov. 4, 845–853.
815 Lawitz, E., Mangia, A., Wyles, D., Rodriguez-Torres, M., Hassanein, T., Gordon, S.C., Schultz, M., Davis,
816 M.N., Kayali, Z., Reddy, K.R., Jacobson, I.M., Kowdley, K.V., Nyberg, L., Subramanian, G.M.,
817 Hyland, R.H., Arterburn, S., Jiang, D., McNally, J., Brainard, D., Symonds, W.T., McHutchison,
818 J.G., Sheikh, A.M., Younossi, Z., Gane, E.J., 2013. Sofosbuvir for previously untreated chronic
819 hepatitis C infection. N. Engl. J. Med. 368, 1878–1887.
820 Lee, E., Pavy, M., Young, N., Freeman, C., Lobigs, M., 2006. Antiviral effect of the heparan sulfate
821 mimetic, PI-88, against dengue and encephalitic flaviviruses. Antiviral Res. 69, 31–38.
822 Li, T.-C., Ami, Y., Suzaki, Y., Takeda, N., Takaji, W., 2013a. No evidence for hepatitis E virus genotype 3
823 susceptibility in rats. Emerg. Infect. Dis. 19, 1343–1345.
824 Li, T.-C., Chijiwa, K., Sera, N., Ishibashi, T., Etoh, Y., Shinohara, Y., Kurata, Y., Ishida, M., Sakamoto, S.,
825 Takeda, N., Miyamura, T., 2005. Hepatitis E virus transmission from wild boar meat. Emerg.
826 Infect. Dis. 11, 1958–1960.
827 Li, T.-C., Suzaki, Y., Ami, Y., Tsunemitsu, H., Miyamura, T., Takeda, N., 2008. Mice are not susceptible
828 to hepatitis E virus infection. J. Vet. Med. Sci. Jpn. Soc. Vet. Sci. 70, 1359–1362.
829 Li, T.-C., Yoshizaki, S., Ami, Y., Suzaki, Y., Yasuda, S.P., Yoshimatsu, K., Arikawa, J., Takeda, N., Wakita,
830 T., 2013b. Susceptibility of laboratory rats against genotypes 1, 3, 4, and rat hepatitis E
831 viruses. Vet. Microbiol. 163, 54–61.
832 Li, W., Sun, Q., She, R., Wang, D., Duan, X., Yin, J., Ding, Y., 2009. Experimental infection of Mongolian
833 gerbils by a genotype 4 strain of swine hepatitis E virus. J. Med. Virol. 81, 1591–1596.

834 Magden, J., Takeda, N., Li, T., Auvinen, P., Ahola, T., Miyamura, T., Merits, A., Kääriäinen, L., 2001.
835 Virus-specific mRNA capping enzyme encoded by hepatitis E virus. J. Virol. 75, 6249–6255.
836 Maila, H.T., Bowyer, S.M., Swanepoel, R., 2004. Identification of a new strain of hepatitis E virus from
837 an outbreak in Namibia in 1995. J. Gen. Virol. 85, 89–95.
838 Mallet, V., Nicand, E., Sultanik, P., Chakvetadze, C., Tessé, S., Thervet, E., Mouthon, L., Sogni, P., Pol,
839 S., 2010. Brief communication: case reports of ribavirin treatment for chronic hepatitis E.
840 Ann. Intern. Med. 153, 85–89.
841 Manns, M.P., Wedemeyer, H., Cornberg, M., 2006. Treating viral hepatitis C: efficacy, side effects,
842 and complications. Gut 55, 1350–1359.
843 Mansuy, J.M., Abravanel, F., Miedouge, M., Mengelle, C., Merviel, C., Dubois, M., Kamar, N.,
844 Rostaing, L., Alric, L., Moreau, J., Peron, J.M., Izopet, J., 2009. Acute hepatitis E in south-west
845 France over a 5-year period. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 44, 74–77.
846 Marschall, M., Stamminger, T., Urban, A., Wildum, S., Ruebsamen-Schaeff, H., Zimmermann, H.,
847 Lischka, P., 2012. In vitro evaluation of the activities of the novel anticytomegalovirus
848 compound AIC246 (letermovir) against herpesviruses and other human pathogenic viruses.
849 Antimicrob. Agents Chemother. 56, 1135–1137.
850 Martin-Serrano, J., Zang, T., Bieniasz, P.D., 2001. HIV-1 and Ebola virus encode small peptide motifs
851 that recruit Tsg101 to sites of particle assembly to facilitate egress. Nat. Med. 7, 1313–1319.
852 Mast, E.E., Alter, M.J., Holland, P.V., Purcell, R.H., 1998. Evaluation of assays for antibody to hepatitis
853 E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. Hepatol.
854 Baltim. Md 27, 857–861.
855 Meng, X.J., Halbur, P.G., Shapiro, M.S., Govindarajan, S., Bruna, J.D., Mushahwar, I.K., Purcell, R.H.,
856 Emerson, S.U., 1998. Genetic and experimental evidence for cross-species infection by swine
857 hepatitis E virus. J. Virol. 72, 9714–9721.

858 Meng, X.J., Purcell, R.H., Halbur, P.G., Lehman, J.R., Webb, D.M., Tsareva, T.S., Haynes, J.S., Thacker,
859 B.J., Emerson, S.U., 1997. A novel virus in swine is closely related to the human hepatitis E
860 virus. Proc. Natl. Acad. Sci. U. S. A. 94, 9860–9865.
861 Meuleman, P., Libbrecht, L., De Vos, R., de Hemptinne, B., Gevaert, K., Vandekerckhove, J., Roskams,
862 T., Leroux-Roels, G., 2005. Morphological and biochemical characterization of a human liver
863 in a uPA-SCID mouse chimera. Hepatol. Baltim. Md 41, 847–856.
864 Miyashita, K., Kang, J.-H., Saga, A., Takahashi, K., Shimamura, T., Yasumoto, A., Fukushima, H.,
865 Sogabe, S., Konishi, K., Uchida, T., Fujinaga, A., Matsui, T., Sakurai, Y., Tsuji, K., Maguchi, H.,
866 Taniguchi, M., Abe, N., Fazle Akbar, S.M., Arai, M., Mishiro, S., 2012. Three cases of acute or
867 fulminant hepatitis E caused by ingestion of pork meat and entrails in Hokkaido, Japan:
868 Zoonotic food-borne transmission of hepatitis E virus and public health concerns. Hepatol.
869 Res. Off. J. Jpn. Soc. Hepatol. 42, 870–878.
870 Moin, S.M., Panteva, M., Jameel, S., 2007. The hepatitis E virus Orf3 protein protects cells from
871 mitochondrial depolarization and death. J. Biol. Chem. 282, 21124–21133.
872 Murakami, Y., Fukasawa, M., Kaneko, Y., Suzuki, T., Wakita, T., Fukazawa, H., 2013. Selective estrogen
873 receptor modulators inhibit hepatitis C virus infection at multiple steps of the virus life cycle.
874 Microbes Infect. Inst. Pasteur 15, 45–55.
875 Nagashima, S., Takahashi, M., Jirintai, S., Tanaka, T., Nishizawa, T., Yasuda, J., Okamoto, H., 2011a.
876 Tumour susceptibility gene 101 and the vacuolar protein sorting pathway are required for
877 the release of hepatitis E virions. J. Gen. Virol. 92, 2838–2848.
878 Nagashima, S., Takahashi, M., Jirintai, Tanaka, T., Yamada, K., Nishizawa, T., Okamoto, H., 2011b. A
879 PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from
880 infected cells. J. Gen. Virol. 92, 269–278.
881 Naik, S.R., Aggarwal, R., Salunke, P.N., Mehrotra, N.N., 1992. A large waterborne viral hepatitis E
882 epidemic in Kanpur, India. Bull. World Health Organ. 70, 597–604.

883 Neukam, K., Barreiro, P., Macías, J., Avellón, A., Cifuentes, C., Martín-Carbonero, L., Echevarría, J.M.,
884 Vargas, J., Soriano, V., Pineda, J.A., 2013. Chronic hepatitis E in HIV patients: rapid
885 progression to cirrhosis and response to oral ribavirin. Clin. Infect. Dis. Off. Publ. Infect. Dis.
886 Soc. Am. 57, 465–468.
887 Neuvonen, M., Ahola, T., 2009. Differential activities of cellular and viral macro domain proteins in
888 binding of ADP-ribose metabolites. J. Mol. Biol. 385, 212–225.
889 Nguyen, H.T., Shukla, P., Torian, U., Faulk, K., Emerson, S.U., 2013. HEV Genotype 1 Infection of Swine
890 Kidney Cells In Vitro is Inhibited at Multiple Levels. J. Virol.
891 Norwitz, E.R., Caughey, A.B., 2011. Progesterone supplementation and the prevention of preterm
892 birth. Rev. Obstet. Gynecol. 4, 60–72.
893 Osinusi, A., Meissner, E.G., Lee, Y.-J., Bon, D., Heytens, L., Nelson, A., Sneller, M., Kohli, A., Barrett, L.,
894 Proschan, M., Herrmann, E., Shivakumar, B., Gu, W., Kwan, R., Teferi, G., Talwani, R., Silk, R.,
895 Kotb, C., Wroblewski, S., Fishbein, D., Dewar, R., Highbarger, H., Zhang, X., Kleiner, D., Wood,
896 B.J., Chavez, J., Symonds, W.T., Subramanian, M., McHutchison, J., Polis, M.A., Fauci, A.S.,
897 Masur, H., Kottilil, S., 2013. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients
898 with unfavorable treatment characteristics: a randomized clinical trial. JAMA J. Am. Med.
899 Assoc. 310, 804–811.
900 Panda, S.K., Ansari, I.H., Durgapal, H., Agrawal, S., Jameel, S., 2000. The in vitro-synthesized RNA from
901 a cDNA clone of hepatitis E virus is infectious. J. Virol. 74, 2430–2437.
902 Parvez, M.K., 2013. Molecular characterization of hepatitis E virus ORF1 gene supports a papain-like
903 cysteine protease (PCP)-domain activity. Virus Res.
904 Patra, S., Kumar, A., Trivedi, S.S., Puri, M., Sarin, S.K., 2007. Maternal and fetal outcomes in pregnant
905 women with acute hepatitis E virus infection. Ann. Intern. Med. 147, 28–33.
906 Perttilä, J., Spuul, P., Ahola, T., 2013. Early secretory pathway localization and lack of processing for
907 hepatitis E virus replication protein pORF1. J. Gen. Virol. 94, 807–816.

908 Pickett, B.E., Sadat, E.L., Zhang, Y., Noronha, J.M., Squires, R.B., Hunt, V., Liu, M., Kumar, S., Zaremba,
909 S., Gu, Z., Zhou, L., Larson, C.N., Dietrich, J., Klem, E.B., Scheuermann, R.H., 2012. ViPR: an
910 open bioinformatics database and analysis resource for virology research. Nucleic Acids Res.
911 40, D593–598.
912 Pischke, S., Hardtke, S., Bode, U., Birkner, S., Chatzikyrkou, C., Kauffmann, W., Bara, C.L., Gottlieb, J.,
913 Wenzel, J., Manns, M.P., Wedemeyer, H., 2013. Ribavirin treatment of acute and chronic
914 hepatitis E: a single-centre experience. Liver Int. Off. J. Int. Assoc. Study Liver 33, 722–726.
915 Pischke, S., Stiefel, P., Franz, B., Bremer, B., Suneetha, P.V., Heim, A., Ganzenmueller, T., Schlue, J.,
916 Horn-Wichmann, R., Raupach, R., Darnedde, M., Scheibner, Y., Taubert, R., Haverich, A.,
917 Manns, M.P., Wedemeyer, H., Bara, C.L., 2012. Chronic hepatitis e in heart transplant
918 recipients. Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg. 12, 3128–
919 3133.
920 Pudupakam, R.S., Kenney, S.P., Córdoba, L., Huang, Y.-W., Dryman, B.A., Leroith, T., Pierson, F.W.,
921 Meng, X.-J., 2011. Mutational analysis of the hypervariable region of hepatitis e virus reveals
922 its involvement in the efficiency of viral RNA replication. J. Virol. 85, 10031–10040.
923 Purcell, R.H., Engle, R.E., Rood, M.P., Kabrane-Lazizi, Y., Nguyen, H.T., Govindarajan, S., St Claire, M.,
924 Emerson, S.U., 2011. Hepatitis E virus in rats, Los Angeles, California, USA. Emerg. Infect. Dis.
925 17, 2216–2222.
926 Purcell, R.H., Nguyen, H., Shapiro, M., Engle, R.E., Govindarajan, S., Blackwelder, W.C., Wong, D.C.,
927 Prieels, J.P., Emerson, S.U., 2003. Pre-clinical immunogenicity and efficacy trial of a
928 recombinant hepatitis E vaccine. Vaccine 21, 2607–2615.
929 Rademacher, C., Guiard, J., Kitov, P.I., Fiege, B., Dalton, K.P., Parra, F., Bundle, D.R., Peters, T., 2011.
930 Targeting norovirus infection-multivalent entry inhibitor design based on NMR experiments.
931 Chem. Weinh. Bergstr. Ger. 17, 7442–7453.

Raj, V.S., Smits, S.L., Pas, S.D., Provacia, L.B.V., Moorman-Roest, H., Osterhaus, A.D.M.E., Haagmans, B.L., 2012. Novel hepatitis E virus in ferrets, the Netherlands. Emerg. Infect. Dis. 18, 1369–1370.

Ratra, R., Kar-Roy, A., Lal, S.K., 2008. The ORF3 protein of hepatitis E virus interacts with hemopexin by means of its 26 amino acid N-terminal hydrophobic domain II. Biochemistry (Mosc.) 47, 1957–1969.

Rehman, S., Kapur, N., Durgapal, H., Panda, S.K., 2008. Subcellular localization of hepatitis E virus (HEV) replicase. Virology 370, 77–92.

Rein, D.B., Stevens, G.A., Theaker, J., Wittenborn, J.S., Wiersma, S.T., 2012. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatol. Baltim. Md 55, 988–997.

Ren, R.B., Costantini, F., Gorgacz, E.J., Lee, J.J., Racaniello, V.R., 1990. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. Cell 63, 353–362.

Rocha-Pereira, J., Jochmans, D., Debing, Y., Verbeken, E., Nascimento, M.S.J., Neyts, J., 2013. The viral polymerase inhibitor 2'-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. J. Virol.

Ropp, S.L., Tam, A.W., Beames, B., Purdy, M., Frey, T.K., 2000. Expression of the hepatitis E virus ORF1. Arch. Virol. 145, 1321–1337.

Sanford, B.J., Dryman, B.A., Huang, Y.-W., Feagins, A.R., Leroith, T., Meng, X.-J., 2011. Prior infection of pigs with a genotype 3 swine hepatitis E virus (HEV) protects against subsequent challenges with homologous and heterologous genotypes 3 and 4 human HEV. Virus Res. 159, 17–22.

Satheshkumar, P.S., Anton, L.C., Sanz, P., Moss, B., 2009. Inhibition of the ubiquitin-proteasome system prevents vaccinia virus DNA replication and expression of intermediate and late genes. J. Virol. 83, 2469–2479.

Schreiber, V., Dantzer, F., Ame, J.-C., de Murcia, G., 2006. Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. 7, 517–528.

Schubert, U., Ott, D.E., Chertova, E.N., Welker, R., Tessmer, U., Princiotta, M.F., Bennink, J.R., Krausslich, H.G., Yewdell, J.W., 2000. Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. Proc. Natl. Acad. Sci. U. S. A. 97, 13057–13062.

Scully, M.F., Weerasinghe, K.M., Ellis, V., Djazaeri, B., Kakkar, V.V., 1983. Anticoagulant and antiheparin activities of a pentosan polysulphate. Thromb. Res. 31, 87–97.

Sehgal, D., Thomas, S., Chakraborty, M., Jameel, S., 2006. Expression and processing of the Hepatitis E virus ORF1 nonstructural polyprotein. Virol. J. 3, 38.

Shrestha, M.P., Scott, R.M., Joshi, D.M., Mammen, M.P., Jr, Thapa, G.B., Thapa, N., Myint, K.S.A., Fourneau, M., Kuschner, R.A., Shrestha, S.K., David, M.P., Seriwatana, J., Vaughn, D.W., Safary, A., Endy, T.P., Innis, B.L., 2007. Safety and efficacy of a recombinant hepatitis E vaccine. N. Engl. J. Med. 356, 895–903.

Shukla, P., Nguyen, H.T., Faulk, K., Mather, K., Torian, U., Engle, R.E., Emerson, S.U., 2012. Adaptation of a genotype 3 hepatitis E virus to efficient growth in cell culture depends on an inserted human gene segment acquired by recombination. J. Virol. 86, 5697–5707.

Shukla, P., Nguyen, H.T., Torian, U., Engle, R.E., Faulk, K., Dalton, H.R., Bendall, R.P., Keane, F.E., Purcell, R.H., Emerson, S.U., 2011. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. Proc. Natl. Acad. Sci. U. S. A. 108, 2438–2443.

Smith, D.B., Purdy, M.A., Simmonds, P., 2013. Genetic variability and the classification of hepatitis E virus. J. Virol. 87, 4161–4169.

Smith, D.B., Vanek, J., Ramalingam, S., Johannessen, I., Templeton, K., Simmonds, P., 2012. Evolution of the hepatitis E virus hypervariable region. J. Gen. Virol. 93, 2408–2418.

Sood, A., Midha, V., Sood, N., 2000. Guillain-Barré syndrome with acute hepatitis E. Am. J. Gastroenterol. 95, 3667–3668.

983 Sugitani, M., Tamura, A., Shimizu, Y.K., Sheikh, A., Kinukawa, N., Shimizu, K., Moriyama, M.,
984 Komiyama, K., Li, T.-C., Takeda, N., Arakawa, Y., Suzuki, K., Ishaque, S.M., Roy, P.K., Raihan,
985 A.S.M.A., Hasan, M., 2009. Detection of hepatitis E virus RNA and genotype in Bangladesh. J.
986 Gastroenterol. Hepatol. 24, 599–604.
987 Sun, X., Bristol, J.A., Iwahori, S., Hagemeier, S.R., Meng, Q., Barlow, E.A., Fingeroth, J.D., Tarakanova,
988 V.L., Kalejta, R.F., Kenney, S.C., 2013. Hsp90 Inhibitor 17-DMAG Decreases Expression of
989 Conserved Herpesvirus Protein Kinases and Reduces Virus Production in Epstein-Barr Virus-
990 Infected Cells. J. Virol. 87, 10126–10138.
991 Suppiah, S., Zhou, Y., Frey, T.K., 2011. Lack of processing of the expressed ORF1 gene product of
992 hepatitis E virus. Virol. J. 8, 245.
993 Surjit, M., Oberoi, R., Kumar, R., Lal, S.K., 2006. Enhanced alpha1 microglobulin secretion from
994 Hepatitis E virus ORF3-expressing human hepatoma cells is mediated by the tumor
995 susceptibility gene 101. J. Biol. Chem. 281, 8135–8142.
996 Takahashi, K., Kitajima, N., Abe, N., Mishiro, S., 2004. Complete or near-complete nucleotide
997 sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients
998 who ate the deer. Virology 330, 501–505.
999 Takahashi, M., Tanaka, T., Takahashi, H., Hoshino, Y., Nagashima, S., Jirintai, Mizuo, H., Yazaki, Y.,
1000 Takagi, T., Azuma, M., Kusano, E., Isoda, N., Sugano, K., Okamoto, H., 2010. Hepatitis E Virus
1001 (HEV) strains in serum samples can replicate efficiently in cultured cells despite the
1002 coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J. Clin.
1003 Microbiol. 48, 1112–1125.
1004 Tamada, Y., Yano, K., Yatsuhashi, H., Inoue, O., Mawatari, F., Ishibashi, H., 2004. Consumption of wild
1005 boar linked to cases of hepatitis E. J. Hepatol. 40, 869–870.
1006 Tanaka, T., Takahashi, M., Kusano, E., Okamoto, H., 2007. Development and evaluation of an efficient
1007 cell-culture system for Hepatitis E virus. J. Gen. Virol. 88, 903–911.

1008 Tanaka, T., Takahashi, M., Takahashi, H., Ichiyama, K., Hoshino, Y., Nagashima, S., Mizuo, H.,
1009 Okamoto, H., 2009. Development and characterization of a genotype 4 hepatitis E virus cell
1010 culture system using a HE-JF5/15F strain recovered from a fulminant hepatitis patient. J. Clin.
1011 Microbiol. 47, 1906–1910.
1012 Tanner, J.A., Zheng, B.-J., Zhou, J., Watt, R.M., Jiang, J.-Q., Wong, K.-L., Lin, Y.-P., Lu, L.-Y., He, M.-L.,
1013 Kung, H.-F., Kesel, A.J., Huang, J.-D., 2005. The adamantane-derived bananins are potent
1014 inhibitors of the helicase activities and replication of SARS coronavirus. Chem. Biol. 12, 303–
1015 311.
1016 Tavassoli, A., Lu, Q., Gam, J., Pan, H., Benkovic, S.J., Cohen, S.N., 2008. Inhibition of HIV budding by a
1017 genetically selected cyclic peptide targeting the Gag-TSG101 interaction. ACS Chem. Biol. 3,
1018 757–764.
1019 Teshale, E.H., Howard, C.M., Grytdal, S.P., Handzel, T.R., Barry, V., Kamili, S., Drobeniuc, J., Okware,
1020 S., Downing, R., Tappero, J.W., Bakamutumaho, B., Teo, C.G., Ward, J.W., Holmberg, S.D., Hu,
1021 D.J., 2010. Hepatitis E epidemic, Uganda. Emerg. Infect. Dis. 16, 126–129.
1022 Tsega, E., Krawczynski, K., Hansson, B.G., Nordenfelt, E., 1993. Hepatitis E virus infection in pregnancy
1023 in Ethiopia. Ethiop. Med. J. 31, 173–181.
1024 Velázquez, O., Stetler, H.C., Avila, C., Ornelas, G., Alvarez, C., Hadler, S.C., Bradley, D.W., Sepúlveda,
1025 J., 1990. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico,
1026 1986-1987. JAMA J. Am. Med. Assoc. 263, 3281–3285.
1027 Versluis, J., Pas, S.D., Agteresch, H.J., de Man, R.A., Maaskant, J., Schipper, M.E.I., Osterhaus,
1028 A.D.M.E., Cornelissen, J.J., van der Eijk, A.A., 2013. Hepatitis E virus: an underestimated
1029 opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation.
1030 Blood 122, 1079–1086.
1031 Volz, T., Allweiss, L., M Barek, M.B., Warlich, M., Lohse, A.W., Pollok, J.M., Alexandrov, A., Urban, S.,
1032 Petersen, J., Lütgehetmann, M., Dandri, M., 2013. The entry inhibitor Myrcludex-B efficiently

1033 blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B
1034 virus. J. Hepatol. 58, 861–867.
1035 Wang, H., He, Y., Shen, Q., Wang, X., Yang, S., Cui, L., Ren, L., Sun, G., Hua, X., Shao, S., Zhang, W.,
1036 2012. Complete genome sequence of the genotype 4 hepatitis E virus strain prevalent in
1037 swine in Jiangsu Province, China, reveals a close relationship with that from the human
1038 population in this area. J. Virol. 86, 8334–8335.
1039 Watashi, K., Inoue, D., Hijikata, M., Goto, K., Aly, H.H., Shimotohno, K., 2007. Anti-hepatitis C virus
1040 activity of tamoxifen reveals the functional association of estrogen receptor with viral RNA
1041 polymerase NS5B. J. Biol. Chem. 282, 32765–32772.
1042 Yamada, K., Takahashi, M., Hoshino, Y., Takahashi, H., Ichiyama, K., Nagashima, S., Tanaka, T.,
1043 Okamoto, H., 2009. ORF3 protein of hepatitis E virus is essential for virion release from
1044 infected cells. J. Gen. Virol. 90, 1880–1891.
1045 Yamashita, T., Mori, Y., Miyazaki, N., Cheng, R.H., Yoshimura, M., Unno, H., Shima, R., Moriishi, K.,
1046 Tsukihara, T., Li, T.C., Takeda, N., Miyamura, T., Matsuura, Y., 2009. Biological and
1047 immunological characteristics of hepatitis E virus-like particles based on the crystal structure.
1048 Proc. Natl. Acad. Sci. U. S. A. 106, 12986–12991.
1049 Yu, C., Boon, D., McDonald, S.L., Myers, T.G., Tomioka, K., Nguyen, H., Engle, R.E., Govindarajan, S.,
1050 Emerson, S.U., Purcell, R.H., 2010. Pathogenesis of hepatitis E virus and hepatitis C virus in
1051 chimpanzees: similarities and differences. J. Virol. 84, 11264–11278.
1052 Zafrullah, M., Ozdener, M.H., Panda, S.K., Jameel, S., 1997. The ORF3 protein of hepatitis E virus is a
1053 phosphoprotein that associates with the cytoskeleton. J. Virol. 71, 9045–9053.
1054 Zeuzem, S., Asselah, T., Angus, P., Zarski, J.-P., Larrey, D., Müllhaupt, B., Gane, E., Schuchmann, M.,
1055 Lohse, A.W., Pol, S., Bronowicki, J.-P., Roberts, S., Arasteh, K., Zoulim, F., Heim, M., Stern,
1056 J.O., Nehmiz, G., Kukolj, G., Böcher, W.O., Mensa, F.J., 2013. Faldaprevir (BI 201335), BI
1057 207127 and ribavirin oral therapy for treatment-naive HCV genotype 1: SOUND-C1 final
1058 results. Antivir. Ther.

1059 Zhao, C., Ma, Z., Harrison, T.J., Feng, R., Zhang, C., Qiao, Z., Fan, J., Ma, H., Li, M., Song, A., Wang, Y.,
1060 2009. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J. Med.
1061 Virol. 81, 1371–1379.
1062 Zheng, Z.-Z., Miao, J., Zhao, M., Tang, M., Yeo, A.E.T., Yu, H., Zhang, J., Xia, N.-S., 2010. Role of heat-
1063 shock protein 90 in hepatitis E virus capsid trafficking. J. Gen. Virol. 91, 1728–1736.
1064 Zhu, F.-C., Zhang, J., Zhang, X.-F., Zhou, C., Wang, Z.-Z., Huang, S.-J., Wang, H., Yang, C.-L., Jiang, H.-
1065 M., Cai, J.-P., Wang, Y.-J., Ai, X., Hu, Y.-M., Tang, Q., Yao, X., Yan, Q., Xian, Y.-L., Wu, T., Li, Y.-
1066 M., Miao, J., Ng, M.-H., Shih, J.W.-K., Xia, N.-S., 2010. Efficacy and safety of a recombinant
1067 hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-
1068 controlled, phase 3 trial. Lancet 376, 895–902.
1069 Zhu, Y., Yu, X., Zhang, Y., Ni, Y., Si, F., Yu, R., Dong, S., Huang, Y., Li, Z., 2013. Infectivity of a genotype
1070 4 hepatitis E virus cDNA clone by intrahepatic inoculation of laboratory rats. Vet. Microbiol.
1071 166, 405–411.
1072

FIGURE LEGENDS

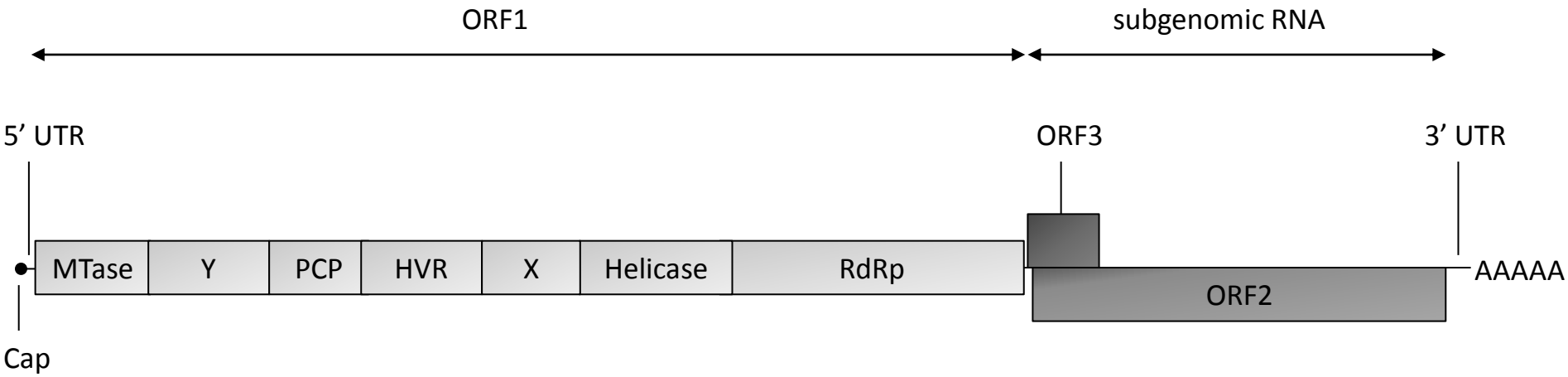
Figure 1 – Organization of the HEV genome: Non-structural proteins are translated from ORF1 while the ORF2 and ORF3 structural proteins are translated from a single subgenomic RNA. UTR, untranslated region; Y, Y-domain; PCP, papain-like cysteine protease; HVR, hypervariable region; X, macro domain; RdRp, RNA-dependent RNA polymerase.

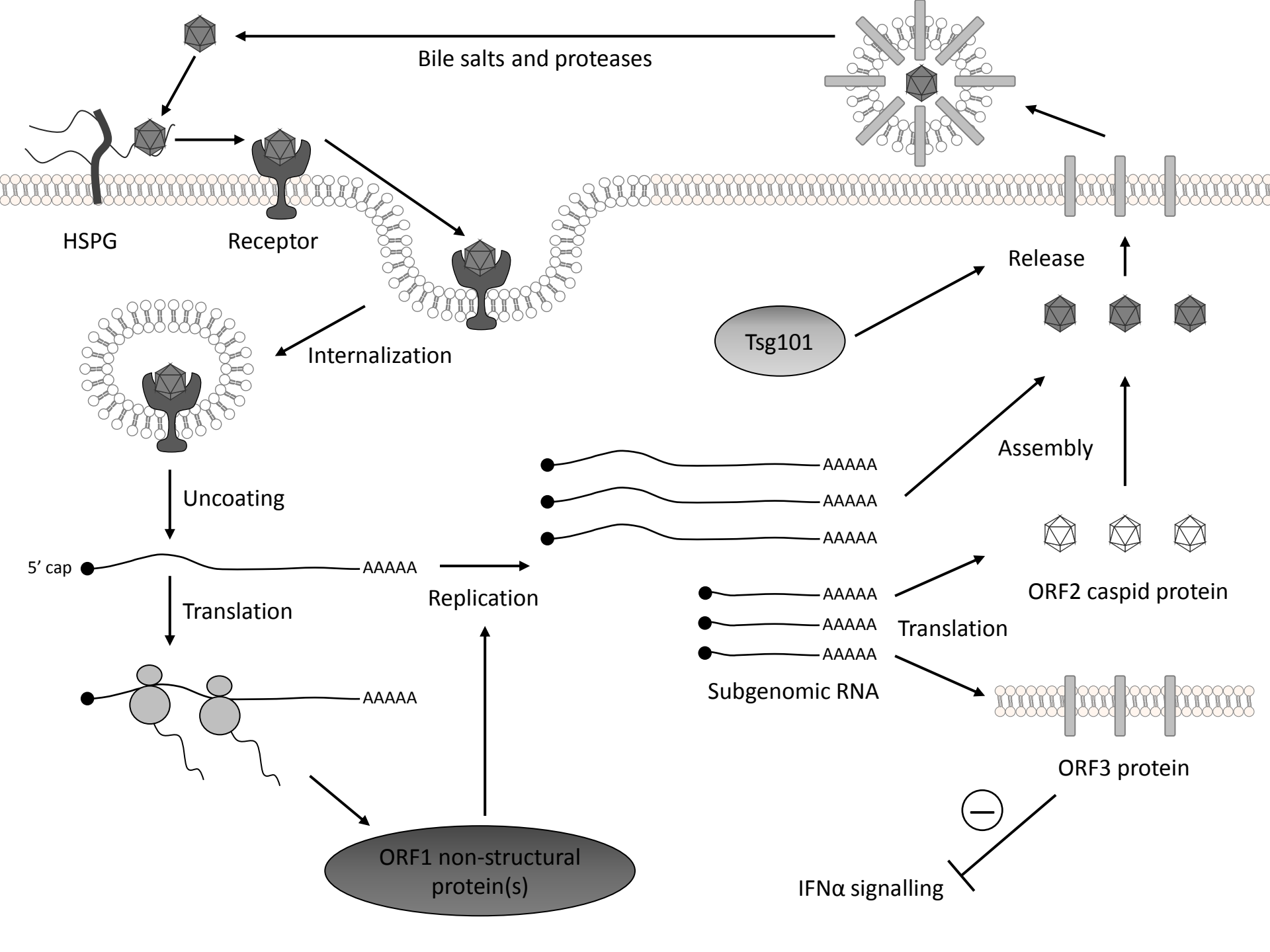
Figure 2 – HEV replication cycle: HEV particles first bind to heparan sulphate proteoglycans (HSPG) and are consequently transferred to an unknown functional receptor, thus mediating cellular uptake through clathrin-mediated endocytosis. Following uncoating, the viral RNA genome is released into the cytoplasm and translated into non-structural proteins. These proteins form a replication complex that produces new full-length and subgenomic viral RNA. The latter is translated into capsid protein (ORF2) and the membrane associated ORF3 protein that is known to interfere with interferon-α signaling. Viral RNA is packaged into capsid protein and released from the cell with help of the host factor Tumor susceptibility gene 101 (Tsg101). The released particles are associated with lipids and ORF3 proteins; both are consequently removed through the bile acids and digestive proteases respectively.

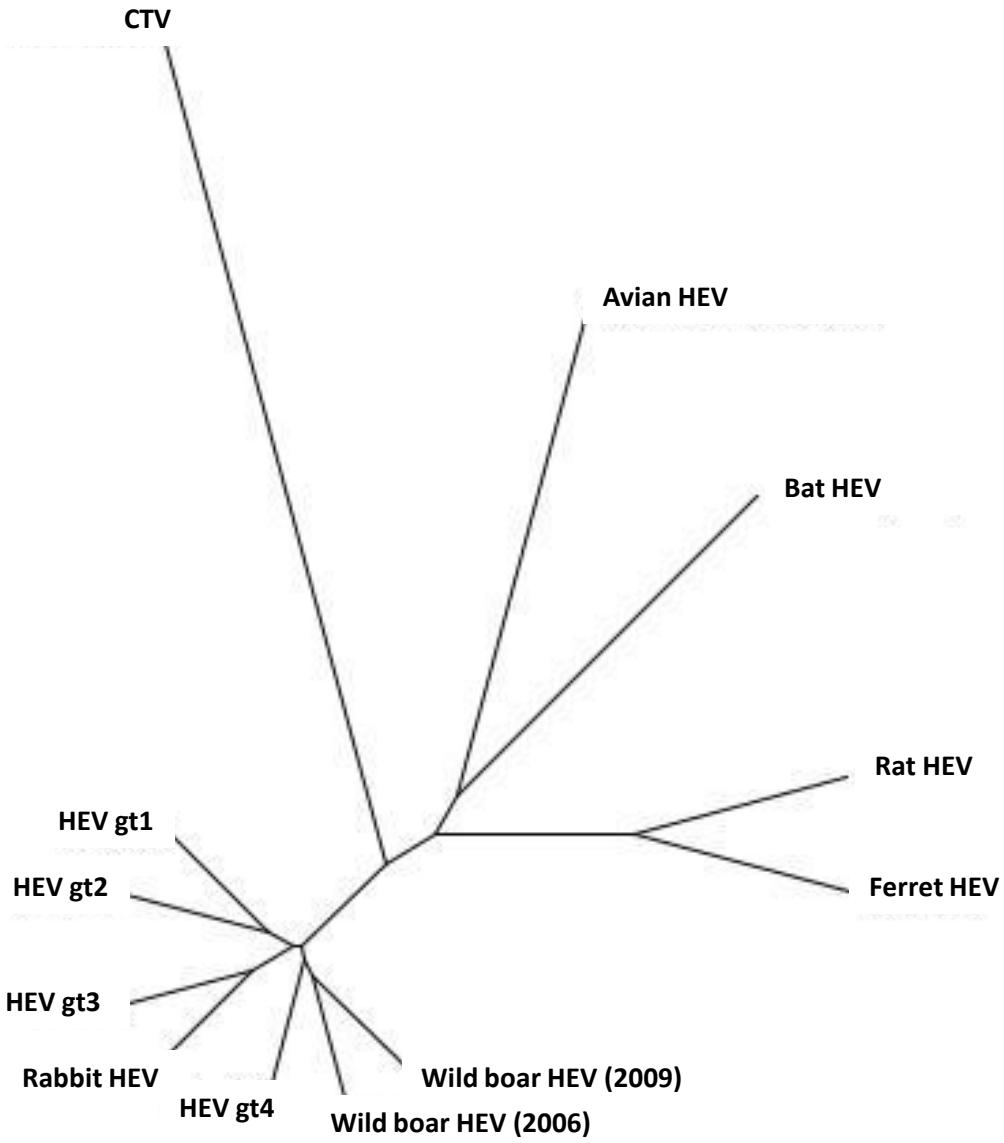
Figure 3 – HEV species and genotypes: The phylogenetic relationship of different human HEV genotypes and the reported animal strains was plotted with the Virus Pathogen Database and Analysis Resource (ViPR, (Pickett et al., 2012)) based on following sequences: HEV gt1 (GenBank accession no. **M80581**), HEV gt2 (**M74506**), HEV gt3 (**HQ389543**), rabbit HEV (**FJ906895**), HEV gt4 (**AB220973**), wild boar HEV 2006 (**AB602441**), wild boar HEV 2009 (**AB573435**), ferret HEV (**JN998606**), rat HEV (**GU345043**), bat HEV (**JQ001749**), avian HEV (**AY535004**), CTV (**HQ731075**). CTV, cutthroat trout virus; gt, genotype.

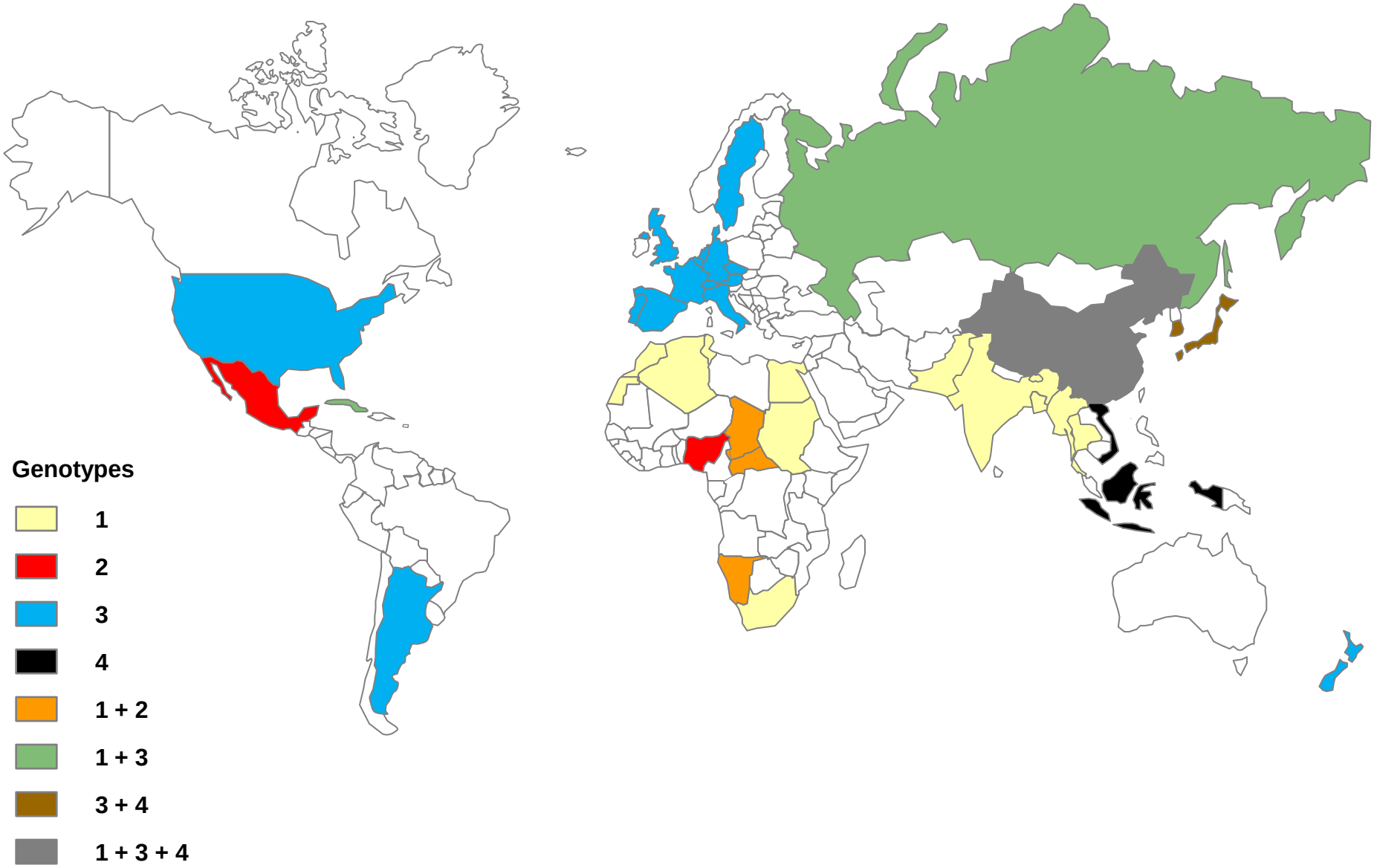
Figure 4 – Geographic distribution of HEV genotypes 1-4: Countries with confirmed human infections are color-coded according to the detected genotype. This is probably an incomplete

picture as there are no data available for many countries and genotyping is not always performed and/or reported.


ORF1
subgenomic RNA
5’ UTR
ORF3
3’ UTR
MTase
Y
PCP
HVR
X
Helicase
RdRp
AAAAA
ORF2
Cap


Bile salts and proteases
HSPG
Receptor
Internalization
Release
Tsg101
Uncoating
5’ cap
AAAAA
Translation
Replication
AAAAA
AAAAA
AAAAA
Assembly
ORF2 caspid protein
AAAAA
AAAAA
AAAAA
Subgenomic RNA
Translation
ORF3 protein
ORF1 non-structural protein(s)
IFNα signalling


CTV
Avian HEV
Bat HEV
Rat HEV
Ferret HEV
HEV gt1
HEV gt2
HEV gt3
Rabbit HEV
HEV gt4
Wild boar HEV (2009)
Wild boar HEV (2006)


Genotypes
1
2
3
4
1 + 2
1 + 3
3 + 4
1 + 3 + 4

Animal model	Virus species	Characteristics	References
Chicken	Avian HEV	Seroconversion, virus shedding, gross hepatic lesions, splenomegaly	(Billam et al., 2005)
Trout	Cutthroat trout virus	Avirulent, only detected during spawning, IFN induction	(Hedrick et al., 1994)
Pig	HEV gt 3 and 4	Seroconversion, virus shedding, histopathological damage	(Feagins et al., 2008; Halbur et al., 2001; Sanford et al., 2011)
Macaque (rhesus, cynomolgus), chimpanzee	HEV gt 1-4	Seroconversion, virus shedding, histopathological damage	(Emerson et al., 2001; Meng et al., 1998; Purcell et al., 2003; Yu et al., 2010)
Rat (Wistar)	Rat HEV	Seroconversion, virus shedding, no alterations in ALT	(Li et al., 2013b)
	HEV gt 1, 3 or 4	Not infectable	(Li et al., 2013b)
(Nude)	Rat HEV	Seroconversion, limited virus shedding, higher titers than in immunecompetent rats	(Li et al., 2013b; Purcell et al., 2011)
	HEV gt 3	Not infectable	(Li et al., 2013a)
(Sprague-Dawley)	Rat HEV	Seroconversion, limited virus shedding, histopathological damage, no alterations in ALT	(Purcell et al., 2011)
	HEV gt 1, 2 or 3	Not infectable (after intravenous injection)	(Purcell et al., 2011)
	HEV gt 4	Seroconversion, virus shedding (after intrahepatic RNA inoculation)	(Zhu et al., 2013)
Mouse (Balb/c nude)	HEV gt 4	Seroconversion, virus shedding, histopathological liver damage	(Huang et al., 2009)
(C57BL/6)	HEV gt 1, 3 and 4	Not infectable	(Li et al., 2008)
Rabbit	Rabbit HEV	Seroconversion, virus shedding	(Cheng et al., 2012)
	HEV gt 4	Seroconversion, virus shedding, symptomatic hepatitis (only for H4-NJ703 strain)	(Cheng et al., 2012)
Mongolian gerbil	HEV gt 4	Virus shedding, slight histopathological liver changes	(Li et al., 2009)

Table 1 – Overview of reported animal models for HEV and surrogate viruses with reported pathology (gt, genotype; IFN, interferon; ALT, alanine transaminase)

HEV-encoded proteins	Confirmed functions	Crystral structure?	References
ORF1	Non-structural proteins		
Methyltransferase	Guanyltransferase, guanine-7-methyltransferase	No	(Magden et al., 2001)
Papain-like cystein protease	Proteolytic processing of ORF1? Deubiquitinating activity	No	(Karpe and Lole, 2011; Parvez, 2013; Perttilä et al., 2013; Sehgal et al., 2006)
Hypervariable region	Influences efficiency of viral RNA replication, host/cell type specificity?	No	(Pudupakam et al., 2011; Shukla et al., 2012)
Macrodomain	Poly-ADP-ribose-binding, weak ADP-ribose 1"-phosphohydrolase activity	No	(Egloff et al., 2006)
Helicase	5'-3' RNA unwinding, NTPase, RNA 5'-triphosphatase	No	(Karpe and Lole, 2010a, 2010b)
RdRp	RNA-dependent RNA polymerase activity	No	(Agrawal et al., 2001; Rehman et al., 2008)
ORF2	Capsid protein	Yes (HEV-like particle)	(Guu et al., 2009; Yamashita et al., 2009)
ORF3	Interaction with multiple cellular proteins, including Tsg101, hemopexin,… Suppression of interferon-α signaling Particle egress	No	(Geng et al., 2013; Ratra et al., 2008; Surjit et al., 2006) (Dong et al., 2012) (Yamada et al., 2009)

Table 2 – HEV-encoded proteins and their (putative) functions